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ANNUAL REPORT OF PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

FISCAL YEAR 1972

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

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NIGMS ANNUAL REPORT

JUNE 1972

CONTENTS

THE DIRECTOR'S REPORT 1

PROGRAM REVIEW

Cellular Basis of Disease 5

Clinical Sciences 32
Trauma
Anesthesiology
Diagnostic Radiology

Engineering in Biology and Medicine 46
Biomedical Engineering 47
Automation of Clinical Laboratories 52

Genetics 60
Pharmacology and Toxicology 79

PROGRAM ADMINISTRATION

Research Grants Branch 93
Research Training Grants Branch 96

THE DIRECTOR'S REPORT

Fiscal year 1972 has been an eventful one for the National Institute of General Medical Sciences. As the Institute nears the anniversary marking its first decade, it is challenged by a world of changes - changes in scientific opportunities, in social concerns, and in Federal perspectives towards institutions and individuals. Recognition of and responsiveness to these challenges are the major responsibilities facing us as Federal science administrators.

Within the special domain encompassed by programs of the NIGMS, the several areas of basic biomedical sciences continue to serve a key underpinning role for the disease oriented programs of the categorical Institutes. This is especially true of the discipline-oriented training grants and fellowships programs which, overall, account for over one-half the predoctorals and almost one-quarter of all the postdoctorals supported by the NIH.

The broad issue of the Federal role in support of graduate research training remains a topic of debate. Congress has been strongly supportive. This year the House-Labor HEW appropriations subcommittee specifically requested that the "Summary Report: Training Programs of the National Institute of General Medical Sciences, 1971-1980", issued September 21, 1971 be included as part of the FY 1973 Budget Hearings. The report, culminating the review of training activities initiated last year appears in Part 4, pp 548-572, of the NIGMS testimony.

The Institute is pleased to announce the launching of the new "Minority Access to Research Careers (MARC) Program", directed toward opportunities for racial/ethnic minority access into biomedical careers. The basic groundwork spanned several years of effort by staff and consultants. In March, 1972, formal announcement was made of the two types of "special" fellowships being offered:

(1) The MARC Faculty Fellowships, to afford advanced research training for minority faculty nominated by their institutions, and
(2) The Visiting Scientist Awards, to strengthen research and teaching programs at minority institutions by drawing upon the talents of expert scientists (such as NIGMS grantees) from other institutions.

Acupuncture

Recent testimonial descriptions of the effects of acupuncture in human patients have been reported by visitors to the People's Republic of China resulting in widespread publicity and public interest. It became evident, on the basis of these factors, that serious attention would be necessary to understand the scientific basis of acupuncture and to determine the role that acupuncture should play in medical care in this country.

Overall responsibility for acupuncture research was assigned by the NIH Director to the NIGMS. Other institutes will obviously be involved in

particular areas. A special advisory committee has been appointed, for which this Institute provides staff support. Dr. Jenerick is acting as executive secretary for this committee, which has scheduled its first meeting in July 1972, to make initial recommendations to the Director, NIH, on a course of action for the NIH.

"Program" Presentations to the NAGMS Council

Continuous efforts are undertaken to bring substantive scientific topics relating to the various NIGMS "programs" before the Council. During FY 72, each Council meeting included a half-day session, each with three to five invited outside speakers. Consonant with the organization of the new NIGMS program area in "Cellular Basis of Disease" a seminar on "Structural and Functional Correlates of Cell Organelles" was presented in November 1971. The Pharmacology/Toxicology program was responsible for a seminar on "Advances in Pharmacology and Toxicology" at the March 1972 Council, and "Applications of Engineering in Biology and Medicine" constituted the seminar in June 1972.

Administrative Aspects

Several staff changes have occurred within my immediate office. Dr. Leo H. VonEuler is now serving as Acting Deputy Director. Dr. Philip S. Chen, Jr., formerly with the Office of the Director, NIH, replaced Dr. Robert E. Greenfield as Asssociate Director for Program Planning and Evaluation. Dr. Trygve Tuve, who died June 1, 1972, served two functions in our Institute, as Associate Director for Manpower, and as Chief, Research Training Grants Branch. These positions have been assumed by Dr. Charles A. Miller. Dr. Howard P. Jenerick and Dr. Vincent E. Price serve as Special Assistants to the Director in addition to other duties.

Organizational Evolvement Towards "Program"Structure

A further step along the lines mentioned in my report last year was the formal designation of a program in "Cellular Basis of Disease." Dr. Vincent E. Price assumed responsibility for administration of this program which encompasses the areas of cell structure and function. This and the Genetics program constitute the two largest program areas from the standpoint of numbers of research grants and total dollars.

Criteria for "Centers"

A growing concern, over the years, with respect to the lack of adequate guidelines for determining the magnitude, growth, funding level, and review of center grants stimulated activity on the part of the NAGMS Council (subcommittee and NIGMS staff to set forth some explicit criteria. The following "characteristics" of NIGMS-supported centers appear to provide a useful administrative guide subject, of course, to modification as our experience with this mechanism of support increases over time.

1. A center is normally multidiscipline and multidepartmental, but it must represent a scientific focus. There should be one cluster of objectives which can be defined and to which answers are being sought.

2. A center will normally be geographically focused. The participating departments and schools will ideally be situated upon a single campus, providing maximal opportunity for collaboration.

3. In a typical center, both basic and clinical departments are expected to be represented.

4. Collaboration among departments is expected to be of a substantial nature. It is anticipated that individual publications arising from a center will frequently show authorship derived from more than one department.

5. Since several departments or schools may be involved, a convincing expression of enthusiam for the center is expected from the higher levels of administration, such as the office of the Dean or of the President of the applying institution.

6. Whereas training stipends will not normally be a portion of the budget of a center, it is confidently expected that most centers will greatly strengthen the training opportunities in the involved departments.

7. Centers will always be limited in number. For this reason, applications will be carefully screened to make certain that the research talents represented are among the highest available in the country.

8. For a center to continue to cohere requires a high level of scientific leadership. Experience has taught us that a center may degenerate into an assembly of isolated research projects in the absence of such leadership. Such deterioration will be an indication for replacement of center grant support by an array of individual project grants.

9. Our experience has indicated that the budget for a center is likely to grow over the first several years of operation as staff and facilities are assembled. However, the hard facts of life preclude growth without limits. Once a center has achieved reasonable size, any further growth will have to be defended on the grounds of special scientific merit. The definition of reasonable size will rest, in part, with our reviewing bodies.

10. A center is construed as a federation of several components. It is expected, as in other federations, that each component

department will happily surrender some degree of autonomy in order to secure a more effective central purpose.

11. The center as a means of support will satisfy certain scientific, institutional, departmental, and personal aspirations. It may conflict with others. Its use should be invoked only when it appears to be the mechanism best suited to fulfill local needs.

Genetics Centers

The Genetics Program is now supporting seven Centers, two of which evolved out of previously funded program projects and five of which are new. These Centers are located at medical centers in California (2), New York (2), Texas, Washington, and Maryland. It is anticipated that additional applications will be reviewed, and possibly funded in the coming fiscal year.

CELLULAR BASIS OF DISEASE

Although research in cell biology has received a great deal of support from the National Institues of Health, it has never had a home. The development of a program on the Cellular Basis of Disease provides for the first time within the NIH--and NIGMS--a central point to which scientists concerned with cell research can turn for program support and for consideration of the special problems and challenges which they face in the decades ahead. Disease may be characterized as a disturbance of normal function or structure of any organ, system, or part of the body. Since the cell is the basic unit of life, all forms and manifestations of disease must ultimately be described in terms of altered structure and function of the cell. For this reason, over the past quarter-century the National Institutes of Health has allocated a considerable fraction of its resources to the acquisition of a more complete and sophisticated knowledge of the cell and its operation. Although much progress has been made, the task is far from finished. Because of its importance to the future of medical science and medical practice, a program on the cellular basis of disease is indeed an appropriate organizational element of the National Institute of General Medical Sciences, which has as its goal the discovery and acquisition of basic knowledge which will have meaning and significance to the programs of all of the categorical Institutes.

Prologue

To lay the foundation for the start of a new program on the Cellular Basis of Disease, this portion of the annual report will depart from the usual submission by devoting its first sections to a survey of the events--both scientific and administrative--which have brought us to this point and to briefly take stock of where we are and of the progress already made in research on the cell and its function.

The history of research on the cell and the cellular basis of disease can be divided conveniently into two periods:

1. The early period, between Hook's discovery of cells in cork in 1664, to 1946--a period of 282 years during which major advances in cell research were made predominantly by European scientists or scientists trained in European laboratories.

2. The recent period, from 1946 to the present--about 26 years--during which the tide of progress in cell research moved to America--largely as the result of increased Federal funding of biomedical research through the NIH extramural programs and through the National Science Foundation.

The Early Period

Although cells were first visualized by Robert Hook in 1664, and his findings confirmed by Malpighi in 1675 and by Grew in plants in 1682, little progress in research on the nature of cells occurred until the

grinding of better lenses by Amici in 1827 led to the development of improved compound microscopes that made possible the remarkable advances in cell research of the 1830's. The cell nucleus was discovered by Brown in 1831, followed by the nucleolus by Valentin in 1836. In 1836-37 Henle described the various forms of epithelia, pointed out that some form of epithelium covers all free surfaces of the body, and published the first treatise on microscopic histology in 1841. In 1838-39 Schleiden proved that plant tissues are made up of groups of cells, and in 1839 Schwann published his classic paper on the cell theory.

During the last half of the 19th and early part of the 20th centuries--largely under support from European governments--histologists and pathologists spent a great deal of time studying living and dead cells from a wide variety of plants and animals in both normal and diseased states. Walther Flemmings' description in 1882 of protoplasm as an "active contractile, net-like material and an inert semi-fluid inter-reticular substance" is as good as any given prior to the modern descriptions of cytoplasm under the electron microscope. We now know, of course, that the inter-reticular substance is far from inert. But then, in 1882 enzymes and proteins were still unknown. The study of dead cells by histological staining methods during this period led to major advances in the diagnosis of disease, and firmly established the causation of disease to events occurring within the cells composing the various organs and tissues of the body. Pathology thus became established as a major medical discipline of vital importance to the development and maintenance of high-quality medical care.

Despite the advances in the study of disease at the organ and tissue level, scientists in 1946 considered the cell to be primarily a "bag of protoplasm" containing a nucleus, and an assortment of granules, vacuoles, and filaments whose structure and function were poorly understood. As late as 1942, protoplasm was defined as "a liquid system of variable viscosity surrounded by an invisible membrane which regulates its interchange with the surrounding medium."[1/] It was further noted that the exact constitution of protoplasm was unknown and that there were many hypotheses as to its structure, including the reticular, fibrillar, granular, and alveolar theories--all of which are now recognized as being, in part, true.

Prior to World War II, leadership in biomedical research came from Europe, from the laboratories of men like Pasteur, Koch, Klebs, and Loffler, in bacteriology; Schwann, Henle, Kolliker, and Flemming, in cytology and histology; and Virchow, the father of pathology. Their exciting findings about the causation of diseases by bacteria and viruses and their descriptions of the nature of cells and tissues and their alterations by disease electrified scientists throughout the world. Scientists from the United States and other countries flocked to Berlin, Vienna, and other universities of Europe to learn the new knowledge being gained. However, with the advent of two world wars during the first half of the twentieth century, medical research efforts in Europe fell to a low ebb.

1/ R. R. Bensley in Maximow and Bloom. Textbook of Histology 1942.

The Modern Period

At the end of World War II, leaders of American medicine and public health, upon surveying the scene, found that most of the serious infectious diseases of man had been alleviated or eliminated; the advent of sulfonamides and antibiotics appeared to be a crowning blow to the large group of bacterial diseases which had plagued man over the centuries. The great health problems facing the nation dealt with serious chronic ailments, such as cancer, arthritis, and heart disease, for which no cure was known or in sight. We lacked the essential base of knowledge from which to proceed; and yet, we could no longer turn to Europe for intellectual leadership. Our own laboratories were poorly staffed and equipped for a major research effort. If we were to learn how to deal with these serious chronic diseases, we needed a much deeper insight as to the underlying nature of disease--and the nature of life itself. The key point is that the major focus for this research effort was the cell--and its detailed structure and function.

With the influx of Federal funds given through the NIH and the National Science Foundation to scientists and universities throughout the nation, major research efforts on the nature of the cell were undertaken, and with these funds it became possible for scientists to develop and acquire sophisticated instruments--such as electron microscopes, ultracentrifuges, spectrophotometers, radio-isotope equipment--that were urgently needed to enable research in molecular and cellular biology to move forward. Fellowships and training grants programs were initiated to enable talented young people to train for careers in this new, exciting research area.

The past quarter century has seen remarkable advances in our knowledge of the cell and its structure and function. It is impossible to present here a detailed account of the progress that has been made. Let it suffice, as an example, to list some of the major structures and organelles now found within the cell and to indicate briefly the state of our knowledge regarding these structures today as compared to 1946.

The Nucleus

As noted previously, the nucleus had first been seen by Robert Brown in 1831, and the nucleolus by Valentin in 1836. Although the process of cell division had been observed by Remak in 1852, chromosomes could not be visualized until biological stains were developed, and in 1882 Flemming gave a classic account of the separation of the chromosomes during mitosis. DNA had been first described by Miescher in 1869, and by 1946 the chemical nature of DNA and RNA had been elucidated in considerable detail. Although scientists knew a great deal about these remarkable biological materials, and suspected their possible role in heredity, this had not been proven--and in 1946 we knew essentially nothing about how the nucleic acids might function in the transfer of biological information from one generation of cells--or organisms--to another, or how this information becomes translated into the structure of the many proteins and enzymes of which each cell is composed. We knew nothing about the genetic code. Since 1953, the profound discoveries of Watson, Nirenberg,

and hundreds of other molecular biologists have given us great insight into the genetic process and have led to one of the most epoch-making periods of discovery of all time.

The Ribosomes

Among the granules that have been discovered within the cytoplasm are the ribosomes. With the assistance and participation of the t-RNA's, these remarkable bodies complete the genetic process by translating genetic information conveyed from the nucleus in the form of messenger RNA into the structure of the proteins and enzymes of the body. Without the ribosomes, the information encoded within the nucleic acids would remain unused. Unknown in 1946, the existence of ribosomes was not even suspected prior to their discovery by biochemists Littlefield, Keller, Gross, and Zamecnik in 1955, who demonstrated their crucial role in protein synthesis. Shortly thereafter, Palade and Siekevitz described their structure as visualized through the electron micoroscope.

The Mitochondria

The mitochondria had been seen in the light microscope by Kolliker in 1857 and were examined by scientists with great curiosity for more than a hundred years. But almost nothing about their detailed structure, or function, was known until the advent of the electron microscope; in 1953 Palade and Sjostrand independently described the basic structural plan of the membranes of the mitochondria. These discoveries, plus the sophisticated biochemical investigations of Lehninger and Green--both NIGMS grantees--and others have provided a remarkable insight into these important organelles and how the enzymes of the electron transport chain and oxidative phosphorylation are organized into highly integrated molecular systems for the conversion of chemical energy from glucose into the high-energy phosphate bonds of ATP, which then provide the energy for muscular contraction and for the wide variety of chemical and metabolic processes required for life.

More recent research has demonstrated the existence of small suborganelles within the mitochondria, which contain the enzymes of the electron transport chain in a highly ordered array. These small bodies, called "electron transport particles" by Green, were first visualized in 1962 by Fernandez-Moran, also an NIGMS grantee.

Lysosomes and Peroxisomes

Among the many different types of vacuoles that had been described within the cell, a considerable body of information has been learned about two groups: the lysosomes, discovered by DeDuve in 1955, which contain powerful digestive enzymes; and the peroxisomes, which contain catalase, d-amino acid oxidase, and other oxidative enxymes. Although relatively little is known about the peroxisomes, a considerable amount has been learned about the lysosomes and the role they play in (1) the digestion and removal of unwanted materials within the cell; (2) the excretion of other materials to the outside of the cell; (3) the sequestering and

storing of materials that cannot be digested; and (4) the controlled removal of unwanted cells during embryonic development. Clinical investigators have shown that the lysosomes play a key role in such diseases as arthritis, silicosis, and other inflammatory diseases.

Myofilaments

The existence of myosin, the contractile protein of muscle and myofilaments, was demonstrated by Edsall and von Muralt in the early 1930's, and actin was discovered in the early 1940's by Szent-Gyorgi. But it was not until the advent of the electron microscope that the detailed structure of muscle could be elucidated, and the existence in other cells of a fine network of myofilaments discerned. There is now great interest in how networks of myofilaments are coordinated to bring about the ameboid motion of cells. Upon seeing electron microscopic pictures of these myofilaments, one cannot help but marvel at Walther Flemming's descriptions of protoplasm in 1882 as an "active, contractile net-like material containing an inert semi-fluid interreticular substance." One only has to remove the word "inert" to render a fairly accurate description of the myofilamentous structure of amoeboid cells as visualized under the electron microscope today.

Microtubules

Microtubules were seen only in rare instances prior to 1963. With the development of gluteraldehyde as a new, gentle fixative for the preparation of cells for electron microscopy, microtubules were seen in most cells and shown to have very unique functions in the separation of chromosomes during cell division, in the mechanism of cell motility by cilia and flagella, and in the maintenance of cell structure--such as the disc-shaped structure of the red blood cell and the long axons of nerves. The detailed structure and function of microtubules is an area of great interest at the present time.

Centrioles

In 1875, Flemming and in 1876, Van Beneden independently discovered a small body lying outside the nucleus that was often paired. This body was called the centrosome by Boverie in 1888 and subsequently found in many other cells and in unicellular organisms. It was regarded as the "dynamic center" of the cell--initiating mitosis by its own division. Although today we know more about the intricate structure of the centriole with its pinwheel-like array of nine triple tubules, we know little more about its function. It has been postulated to be an organizing or assembly center for the formation of the microtubules needed for cell division; however, plant cells have no centrioles, but do undergo mitosis. Although the role of centrioles in cell division is in question, their role as the precursors of the "basal bodies" necessary for the formation of flagella and cilia appears to be mandatory. We need to know much more about centrioles and their possible function as organizing centers for the formation of microtubules within the cell.

The Cell Membrane and Endomembrane System

Although the existence of a cell membrane was inferred for many years from our knowledge of the permeability characteristics of cells, the cell membrane *per se* could not be visualized prior to the development of the electron microscope. Its presence was first described by Fernandez-Moran in the Schwann cells surrounding nerve fibers. In the ensuing years, a great deal of research has been conducted in an effort to understand and elucidate the true nature of the cell membrane. The unit membrane hypothesis of Robertson (1951), although being vigorously defended, is also under assault from all sides, and the true nature of the membrane thus may take a number of years to unravel. However, unknown to cell biologists prior to 1946, we now have a view of the external cell membrane as being continuous with, and possibly the precursor of, an elaborate network of membranes called the "endomembrane system" within cells. These membranes include: (1) the *peri-nuclear membrane*, which surrounds the nucleus and contains a large number of pores that may permit the passage of molecules into and out of the nucleus; (2) the *endoplasmic reticulum*, described by Porter in 1953; (3) the *Golgi apparatus*, described by Golgi in 1898; and (4) the membranes surrounding the lysosomes, discovered by DeDuve in 1955. Evidence has been presented of a continuity between the various cell membranes, thus indicating theoretically a continuous pathway between the nucleus to the outer cell wall through the passages of the endomembrane system, in a manner that does not involve direct contact with the cytoplasm. The endomembrane system has been explored at length by Palade and others; its most obvious function appears to relate to protein synthesis by ribosomes at sites along the "rough" endoplasmic reticulum (ER). The newly synthesized protein is transferred through the wall of the ER into the lumen, modified in the Golgi apparatus by addition of carbohydrate moieties, and finally excreted to the outside, either directly or through membrane-lined packets similar or identical to the lysosomes. Although a general picture of the endomembrane system has been formulated during the past score of years, our knowledge of this important system is still in a relatively primitive state.

Proteins and Enzymes

Although the achievements listed above are remarkable, they are only symbolic of the vast amount of knowledge that has been gained about the constituents of cells. Thus although the role of enzymes as catalysts of highly specific biochemical reactions was demonstrated by Fischer in 1894 and the polypeptide nature of proteins was described by Fischer and Hoffmeister (1902), it was not until 1926 that Sumner proved that the enzyme urease was a protein. Sumner's reports were ridiculed until Northrup in 1930-36 isolated and crystallized three digestive enzymes--pepsin, trypsin, and chymotrypsin. In the intervening years, more than a thousand enzymes have been described and their metabolic role investigated. More than 150 enzymes have been crystallized. In 1959, Gundlach, Moore, and Stein demonstrated the presence of an "active center" within the enzyme ribonuclease, and in 1963, Moore and Stein completed their determination of the linear sequence of the amino acids in the enzyme. During the years 1961-63, Anfinsen at NIH showed that forces imposed on the

linear chain of ribonuclease by polar and nonpolar side chains of the amino acids within the sequence caused the chain to spontaneously fold so that polar groups were exposed to the aqueous exterior, and nonpolar groups were turned into an anhydrous interior. Thus for the first time we were able to see how the linear chains of amino acids produced by the ribosome--from linear RNA messages sent from the nucleus--fold into the complex three-dimensional enzyme structures. At the present time a great deal of effort is being expended by biophysicists and biochemists in the determination of the three-dimensional structure of a wide array of enzymes--and in research on the nature of their active centers--in an effort to determine how they perform their complex chemical work with effortless simplicity. Upon knowing the structure of an enzyme, they are beginning to learn how the activity of an enzyme can be regulated and modified by conformational changes within the proteins brought about by ions, drugs, hormones, or other substances.

How Does Cell Research Relate to Human Health?

1. The remarkable advances in bacteriology, histology, pathology made in European laboratories during the last half of the nineteenth century provided the scientific base for the practice of medicine during the first 60 years of the twentieth century. Similarly the advances of the past quarter-century--the elucidation of the biochemical basis of genetics, the unraveling of the complex metabolic pathways of the cell, the detailed description of the fine structure of the mitochondria, and the discovery of many new cell organelles that were completely unknown in 1946--can be expected to establish a whole new base for the practice of medicine in the late twentieth and early twenty-first centuries. It can be fully expected that our knowledge of the structure and function of cell organelles such as the mitochondria, microtubules, lysosomes, and endoplasmic reticulum will be as important to the practice of medicine over the next 75 years as our knowledge of the heart, lungs, liver, and kidneys is to the medicine of today. The advances in cell research that have been made since 1946 are landmark discoveries, and the NIH--and the nation--can be proud of the role they have had in this enormously fruitful effort.

2. The proteins and enzymes of the body are the products of man's genes. Errors in the genetic constitution of man that arise as the result of mutation are reflected in the structure of the proteins and enzymes of his body. The techniques of protein and enzyme chemistry that have been developed are enabling man to understand the consequences of genetic mutation and to study and explore possible means of alleviating the conditions thus created. Thus sickle cell anemia arises as the result of the substitution of the amino acid valine for glutamic acid in position 6 of the beta chain of hemoglobin. The alpha chains are normal. The loss of the acidic residue of glutamic acid in position 6 leads to increased hydrogen bonding between the hemoglobin molecules and their stacking into semi-rigid, microtubular-like structures and the loss of plasticity of the red cells, occlusion of capillaries, and the symptoms of sickle cell anemia. Recent studies, in 1971, by Cerami and Manning in Moore and Stein's laboratory, carried out under grant GM 07256-13, suggest that the attachment of a

cyanate group on the terminal amino group provides the needed acidic residue, thus preventing the stacking of hemoglobin molecules and lessening the symptoms of sickle cell anemia. Thus knowledge of protein chemistry may help to minimize or lessen the debilitating effects of sickle cell anemia.

3. Although we have a large amount of empirical knowledge of the ways in which drugs act upon the body, we have only a primitive knowledge of how they act at the molecular and cellular level. It is quite conceivable, for example, that many drugs act to modify the structure and conformation of proteins and enzymes. Detailed knowledge of the structure of proteins and enzymes may enable us to discover how many drugs act and help us to tailor and synthesize drugs for a specific action at a given locus on a given protein or enzyme within the body. This might well be called submolecular pharmacology and is an area of great importance for the future.

4. Although the pay-off for medicine may be great, the pay-off for mankind as a society may be equally important. Man's technological innovations have outpaced his capacity to deal with their products. Whereas man's factories pollute the air and waters with highly toxic chemicals, the chemical factories of the body--the enzymes--often do similar work with great ease at normal temperatures and without the use of highly toxic reagents at high temperatures necessitating large expenditures of energy. Can it be that the development of fundamental knowledge regarding nature's catalysts--the enzymes--may enable man to develop new technologies that will enable him to design, develop, and manufacture new enzymes tailored to do specific catalytic functions in factories designed and operated by man?

To view it in a different way, the ribosome may be two billion years old, and man two million years old--but man has known of the existence of the ribosome for less than 20 years. The ribosome has built into it a "molecular technology" and "know-how" perfected by principles of natural selection through trillions of experiments over the billions of years that life has been on earth. When life scientists, through fundamental research, learn this molecular technology, they will not only understand the nature of life, and of disease, but will also have in their hands the capability of using this knowledge to meet pressing industrial and social problems--such as pollution--in ways that may make present technology primitive indeed.

Development of Cell Research at the NIH

During the earlier years of the extramural programs at the NIH, research in cell biology and genetics was supported largely as an adjunct to the programs of the several Institutes, with each Institute supporting those areas of basic sciences pertinent to its categorical mission. This approach was aided by the fact that for much of the early period one Institute--Experimental Biology and Medicine--remained fairly non-categorical and oriented to the broader aspects of biology and medicine. When this Institute was renamed the National Institute of Arthritis and Metabolic

Diseases in 1950, it lost some of its non-categorical character. During the succeeding years, a number of grants of high scientific priority accumulated that could not be readily assigned to any of the Institutes. For several years, these grants were funded through and administered by the Division of Research Grants.

It became increasingly apparent that it would be highly desirable and advantageous to develop an administrative unit within the NIH oriented specifically to the basic sciences. This led in 1958 to the creation of the Division of General Medical Sciences, and to its subsequent transformation into the National Institute of General Medical Sciences in 1963. The primary task of the new Institute was to support research and training in the basic medical sciences and, in addition, to support those areas of the medical sciences that were not within the domain of the categorical Institutes (including the clinical areas of surgery, anesthesiology, and diagnostic radiology). With increased emphasis by the Congress and the Administration on short-range, applied research, special programs were evolved during the late 1960's in pharmacology-toxicology, biomedical engineering and automated clinical laboratories, and in trauma research. The bulk of the basic sciences, including cell structure and function, remained grouped under a large general category called "Fundamental Sciences," with considerable dollar support, but little special attention on the part of the top staff of the Institute.

The point of the above is to emphasize the rather diffuse approach to the support of the basic sciences that characterized the earlier years--due largely to the heavy categorical and applied emphasis of NIH funding through the Congress. Despite the lack of specific attention, the basic sciences have, in general, prospered, and many of the advances in cell research since 1946 described above have come as the result of general support given to them by all of the Institutes of the NIH, as well as by the National Science Foundation.

Distribution of Cell Research Among the Institutes

The appropriate distribution of support for cell research among the Institutes is shown in Table 1-A and 1-B. This table was developed by assuming that all of the research grants under support by the given Institute that had been reviewed by the Biophysics and Biophysical Chemistry A and B, Biochemistry, Bacteriology and Mycology, Cell Biology, Microbial Chemistry, Molecular Biology, and Physiological Chemistry Study Sections were directly pertinent to cell research (Table 1-A); and that a significant fraction[2/]--as shown in parentheses in Table I-B--of the research reviewed by the Genetics, Immunobiology, and Pathology A and B Study Sections is also considered to be cell research. Lesser amounts of cell research under support by the Institutes but reviewed by other study sections (e.g., 20 percent of the Physiology and 10 percent of the Metabolism Study Sections) have been omitted.

2/ Determined by the Executive Secretaries of those Study Sections.

Table I

NIH SUPPORT OF MOLECULAR AND CELLULAR BIOLOGY
Dollars Awarded - FY 1972

A. Program Areas Dealing Solely With Molecular and Cellular Biology

Study Sect.	NIAID	NIAMDD	NCI	NIDR	NIEHS	NEI	NIGMS	NICHD	NHLI	NINDS	DRR	Total by S.S.
BCA	149,391	1,323,601	1,136,683	37,176	34,497	440,792	4,682,666	45,564	929,871	247,592	---	9,027,833
	4	*32*	*27*	*1*	*1*	*12*	*87*	*1*	*25*	*7*	---	*197*
BCB	376,723	2,170,315	618,905	36,353	---	156,999	5,841,962	32,275	1,576,263	511,129	510,786	12,831,710
	7	*52*	*11*	*1*	---	*6*	*112*	*1*	*39*	*17*	*4*	*250*
IO	638,706	3,966,729	2,992,107	28,566	---	55,042	5,442,955	282,920	901,180	555,175	134,780	14,998,160
	12	*95*	*74*	*1*	---	*3*	*116*	*5*	*21*	*14*	*1*	*342*
	7,701,470	78,372	16,229	155,163	34,255	36,188	199,182	197,786	183,270	---	---	8,601,915
	192	*2*	*1*	*5*	*1*	*1*	*4*	*7*	*6*	---	---	*219*
BY	151,621	921,540	1,427,668	116,654	76,751	106,058	2,285,342	2,231,076	452,147	1,017,238	---	8,786,095
	5	*25*	*32*	*5*	*2*	*4*	*54*	*54*	*12*	*26*	---	*219*
BC	4,620,329	1,306,157	564,322	36,272	107,221	---	2,215,647	166,334	20,901	44,428	20,000	9,101,611
	115	*40*	*16*	*1*	*3*	---	*60*	*5*	*1*	*2*	*1*	*244*
BY	661,042	1,625,260	2,206,356	---	---	---	3,193,428	1,464,122	501,621	615,030	---	10,266,859
	10	*38*	*51*	---	---	---	*62*	*27*	*14*	*18*	---	*220*
C	1,204,618	8,364,786	2,315,091	197,194	---	146,286	5,812,409	1,046,550	2,266,152	1,860,225	---	23,213,311
	23	*192*	*45*	*6*	---	*4*	*102*	*26*	*44*	*48*	---	*490*
Total	15,503,900	19,756,760	11,277,361	607,378	252,724	941,365	29,673,591	5,466,627	6,831,405	4,850,817	665,566	95,827,494
	368	*476*	*257*	*20*	*7*	*30*	*537*	*126*	*162*	*132*	*6*	*2,141*

B. Program Areas Dealing Partially or Significantly With Molecular and Cellular Biology

Study Sect.	NIAID	NIAMDD	NCI	NIDR	NIEHS	NEI	NIGMS	NICHD	NHLI	NINDS	DRR	Total by S.S.
EN (60%)*	2,973,118	426,560	1,623,267	24,717	163,179	---	8,729,035	2,104,931	330,191	242,026	12,884	16,629,908
	55	*9*	*24*	*1*	*3*	---	*184*	*41*	*8*	*8*	*1*	*334*
MB (60%)*	6,385,937	2,153,708	3,106,552	28,700	---	73,256	228,106	476,576	321,275	515,922	48,840	13,338,873
	121	*49*	*56*	*2*	---	*2*	*3*	*10*	*6*	*11*	*1*	*261*
THA (40%)*	622,146	2,066,899	537,417	8,474	27,218	---	152,200	238,142	2,868,256	1,475,566	---	7,996,318
	19	*58*	*15*	*1*	*1*	---	*4*	*8*	*69*	*37*	---	*212*
THB (40%)*	326,499	1,412,812	6,573,708	50,031	339,920	---	179,868	163,545	806,501	3,216	---	9,856,100
	8	*27*	*139*	*1*	*8*	---	*2*	*3*	*14*	*1*	---	*203*
Total	10,307,700	6,059,979	11,840,944	111,922	530,317	73,256	9,289,209	2,983,194	4,326,224	2,236,730	61,724	47,821,199
	203	*143*	*234*	*5*	*12*	*2*	*193*	*62*	*97*	*57*	*2*	*1,010*

*Estimated Fraction
No. Programs in Italics.

It will be seen in Table I-A that the grants reviewed by the eight listed study sections that are now under support by the NIH total about $96 million, of which NIGMS has about $30 million, NIAMDD $20 million, NIAID $16 million, NCI $11 million, and lesser amounts for other areas. Among the disciplines listed, NIGMS supports about half of biophysics and genetics, a third of biochemistry and molecular biology, a quarter of cell biology, microbial chemistry, and physiological chemistry, and about two percent of bacteriology and mycology, immunobiology, and pathology.

Development of a Program on the Cellular Basis of Disease in NIGMS

Following his appointment in October 1970, the new Director of NIGMS, after careful examination of the programs of the Institute, felt that the fundamental sciences merited the same special attention on the part of staff that had previously been accorded the clinical and applied sciences. He further recognized that a large and undifferentiated program in the fundamental sciences--with an emphasis on granting mechanisms (i.e. research grants, training grants, and fellowships) rather than on scientific content--made it difficult to attract and hire the quality of staff needed to move these vitally important programs ahead. He therefore determined that progressively the grants of the fundamental sciences areas should be assigned to specific program areas. First priority was given to genetics, which has received prominent attention during the past 18 months. The next large area requiring special attention pertains to those grants in the fundamental sciences related to research on cell structure and function, which for program purposes is called the Cellular Basis of Disease. This leaves two smaller areas--bio-related chemical processes, and adaptation--for future attention.

The present status of research grant support among the NIGMS programs is shown in Table II. Three of the programs under Fundamental Sciences--Genetics and Genetic Chemistry, Cell Structure and Function, and Bio-related Chemical Processes--are aimed at the acquisition and discovery of new knowledge that will provide a greater understanding of the molecular and cellular basis of disease, and might well be grouped together under this general term, particularly since close coordination of both research and training efforts is essential. In FY 72, the research efforts among these three programs included 1,004 grants totaling $60.8 million, or 64 percent of the Institute's research grant support. These 1,004 grants averaged $60,550 per grant.

Training in Molecular and Cellular Biology

At the predoctoral level, the NIGMS has a broad and important training role with the function of providing trained basic scientists for all of the categorical Institutes, as well as for its own research programs. Traditionally, these programs have been oriented to the basic science disciplines found in medical school and university graduate programs. Training programs heavily involved in cell research include the following: Anatomical Sciences, Biochemistry, Biophysical Sciences, Genetics, Microbiology, Pathology, and Multidisciplinary Biological Sciences. As shown in Table III, training in these areas in 1972 amounted to approximately $21 million, or almost 50 percent of the training budget. In addition, a considerable fraction of the Institute's fellowship and career award programs are devoted to the

Table II

NIGMS Research Grants
By Program Area
FY 1972

Program	Number Grants	Amount	% of Total $
Fundamental Sciences			
a. Genetics and Genetic Chemistry	463	$31,976,907	33.8
b. Cell Structure and Function	401	22,774,001	24.0
c. Bio-Related Chemical Processes	140	6,042,609	6.4
d. Adaptation and Behavior	14	1,573,043	1.8
Total	1,018	62,366,560	66.0
Pharmacology and Toxicology	62	10,219,771	10.8
Engineering in Biology and Medicine			
a. Biomedical Engineering	41	5,871,207	6.2
b. Automation of the Clinical Laboratory	45	4,529,852	4.8
Total	86	10,401,059	11.0
Clinical Sciences			
a. Anesthesiology	15	4,437,604	4.7
b. Diagnostic Radiology	19	2,761,251	2.9
c. Trauma	30	3,423,967	3.6
d. Clinical Medicine	12	925,710	1.0
Total	76	11,548,532	12.2
TOTAL RESEARCH GRANTS	1,242	$94,535,922	100.0%

VEPrice
10/30/72

Table III

NIGMS Support of Manpower
Related to Cell Research

Dollars (In Thousands)

Program	Training Grants	Fellow-ships	Career Awards	Total
Anatomical Sciences	$ 1,925	$ 99	$ 149	$ 2,173
Biochemistry	5,309	1,366	2,863	9,538
Biophysical Sciences	2,756	960	1,202	4,918
Genetics	2,863	1,732	1,395	5,990
Microbiology	2,419	370	299	3,088
Multidisciplinary Biological Sciences	2,158	--	777	2,935
Pathology	3,530	103	57	3,690
Total	20,960	4,630	6,742	32,332

support of individuals doing training and research in these areas; these data are also shown in Table III.

Fraction of the Institute Programs Related to Cell Research

It will be seen from Tables II and III that $60.8 million in research grants and $32.3 million in training grants, fellowships, and career awards are devoted to programs related primarily to research in molecular and cellular biology. Thus, approximately $93 million out of $154 million expended by the Institute through its extramural programs is devoted to cell research. (It should be noted that these data are only approximate, since certain segments of genetics, anatomical sciences, and pathology are for research and training at a level above the cell. On the other hand, parts of the pharmacology-toxicology, medicinal chemistry and automated clinical laboratory programs are devoted to research and training at the molecular and cellular level. Therefore, the level of effort devoted to cell research is 60 percent, as a first approximation, of the Institute's grants and fellowship programs.

Boundary Lines Between Programs

It is impossible to completely separate training devoted to genetics from that related to cell structure and function. The enzymes and proteins of the cell are the products of genetic information, and no modern molecular or cellular biologist should be trained solely in genetics or in cell structure and function. With regard to research, however, the sheer size of the programs makes a logical division between the programs necessary; a tentative boundary that will be used is as follows:

The genetics program includes those research areas related to all aspects of the genetics process, including research on the cell nucleus, on chromosomes, and on the ribosomes, as well, since they are involved in genetic expression, and on the nucleic acids and their constituents. This program also includes all aspects of the Institute's research and training efforts with regard to the alleviation and/or cure of genetic disease.

The cellular basis of disease program starts with the linear protein chain upon its release from the ribosome and includes all aspects of the folding of the linear chain into functional proteins and enzymes, the aggregation and organization of these proteins and enzymes into membranes or other functional units, the action of enzymes in metabolic cycles within the cell, the structure and function of cell organelles other than the nucleus and the ribosome, cell division and the cell life cycle, and the regulation and control of nongenetic events within cells.

It is realized that these assignments are somewhat arbitrary and that in certain areas--especially in regulation and control--an overlapping effort that bridges genetics and cell structure and function may be required. These areas are being examined and considered carefully as the two programs develop in parallel.

Organization of Cellular Basis of Disease

The genetics program is discussed in a following section of this report. The program dealing with the cellular basis of disease can be examined and organized in a number of ways:

A. Organization by scientific discipline; this closely approximates the study section designations to which the grant application was assigned for review. By this classification, most grants can readily be placed in one of three groups:

1. Biophysics and biophysical chemistry: research on the structure of proteins and other macromolecules, and their organization into membranes and cell organelles.

2. Biochemistry: research on enzymes and their organization into metabolic pathways and cycles within the cell; their regulation and control; microbial biochemistry.

3. Cell biology: research on whole cell and cell organelles, cell life cycle and the coordination of events within cells, cell-cell recognition and communication, cell differentiation and specialization.

This organization has the advantages of administrative simplicity in that it provides a rather direct 1:1 relationship between program staff and study section personnel, on the one hand, and the Institute's research training grants staff on the other--except that there is no single training program responsible for the area of cell biology; this activity is shared by the training programs in microbiology, the anatomical sciences, pathology, and multidisciplinary biology.

B. Organization according to traditional categories of research:

1. Proteins: research on the linear sequence and three-dimensional structure of proteins.

2. Enzymology: studies of the active sites of enzymes in relation to their function.

3. Metabolism: the functioning of enzymes in metabolic pathways and cycles to carry out the cell's biochemical activities.

4. Organelles: the study of the structure and function of cell organelles.

5. Membranes and ion transport: research on the origin, structure, and continuity of membranes and the mechanism by which they create and regulate localized environments within cells; ion and amino acid transport; receptor sites.

6. Whole cells: research on whole cells and their life cycle; coordination of events within cells; methods for their culture.

7. Differentiation: investigations on the sequence of events leading to the differentiation of cells into highly specialized forms.

8. Methods: developmental research on new methods, instruments and approaches to investigations on cells and their constituents.

The estimated distribution of research effort among these traditional research areas for FY 72 in shown in Table IV.

C. Organization by concepts and problem areas:

1. Macromolecular structure: physical chemistry and structure of proteins and other macromolecules.

2. Catalysis: studies on the relationship of the three-dimensional structure of enzymes to their catalytic properties.

3. Metabolic transformations: enzymatic and chemical processes involved in the synthesis and interconversion of biochemicals required for life processes.

4. Bioenergetics: physico-chemical and biochemical events in the production of energy; photosynthesis.

5. Tranduction of energy into useful biological forms:
   mechanical--contractility of muscle, cilia, and flagella;
   chemical--for synthetic processes within cells;
   electrical--for ion transport through membranes; biocommunication.

6. Compartmentalization: to maintain and regulate highly specialized environments within cells.

7. Translocation and transport: movement of materials through cells; translocation from one compartment to another; transport from inside to outside and vice versa.

8. Recognition and communication: receptor sites on cell surfaces; intra- and inter-cellular communication, chemotaxis and other sensory processes of cells; biological recognition of proteins and other macromolecules; mechanisms of inspection and quality control at both molecular and cellular levels.

9. Regulation and coordination of events within cells; regulation of enzymes and metabolic pathways; coordination of cell movement in response to sensory processes.

10. Differentiation and specialization: cell transformations in response to genetic control, enzyme induction, integration of genetic and other biochemical events leading to cell specialization.

Table

CELL STRUCTURE & FUNCTION

FY 1972 (Estimated)

CLASSIFICATION	# OF AWARDS	$ (IN MILLIONS)	%
Proteins	63	$3.68	16.1
Enzymology	82	4.82	21.1
Metabolism	98	5.39	23.6
Organelles	38	2.39	10.5
Membranes and Ion Transport	43	1.97	8.6
Whole Cells	11	0.79	3.5
Differentiation	22	0.77	3.4
Methods	44	2.96	13.0
Totals	401	22.77	100.0

11. Cell life cycle: sequential steps in cell division and the subsequent replication of new membranes and organelles required for the growth of daughter cells.

12. Limiting events in the growth and survival of cells, responses of cells to mechanical and chemical injury, aging and senescence, mechanisms of cell death.

This organization is the most interesting, but administratively the most complex.

It is planned that at least for the coming year Alterative A, organization by scientific discipline, will be used for structuring the activities of program personnel, because it provides the simplest organizational scheme for coordinating and cross-relating the activities of the research, training, and fellowships branches, and also those of DRG. However, increasingly for reporting purposes, scientific progress in the conceptual problem areas will be presented as described in Alternative C--since this is intellectually the most satisfying.

Programmed versus Unprogrammed Research

Because of its essential root in the fundamental sciences, this--of all the NIGMS programs--has been most dependent upon highly innovative, investigator-initiated research subjected to rigorous review by study sections or other initial review groups. It is expected that this process of continued support of investigator-initiated projects based on excellence, as judged by highly qualified peer groups, will continue to form the mainstay for this program.

Centers versus Grants

Centers have certain definite values in promoting common effort between disciplines and research areas where cooperative programs are needed but hard to achieve. Centers also provide readily identifiable foci where a group of investigators may be working together to fulfill an important national need, as in pharmacology and toxicology. A description of the general characteristics that will be looked for in the development of centers is found in the Director's Report.

It is expected that unless specific funds for centers in cell research are forthcoming from the Congress the development of a centers program in this area will not be rapid, but will be highly selective and develop gradually over several years.

One area of urgent and considerable concern to the Institute is to bring about a more rapid movement of the burgeoning knowledge of genetics and cell structure and function into pathology. Just as the research in the pathology of organs and tissues of a century ago led to the medicine of the twentieth century, research in the pathology of organelles, membranes, and other subcellular structures will almost certainly provide the basis for the medicine of the twenty-first century. If research in pathology is to move forward, it must be solidly based on the remarkable advances in molecular and cellular

biology that we have witnessed over the last score of years. The Institute will do all that it can within the limitations of available funds to bring this about.

Research Highlights

Research Highlights are the most difficult part of the report to write for the following reasons:

1. Research findings that are new are frequently not proven; those which are proven are usually not new.

2. Progress by any given investigator in a single year, although it may be very important in the step-by-step attack on a given scientific problem, usually seems insignificant in the "big picture." A period of 4-5 years may be needed to show progress that appears significant from the perspective of one viewing the total biomedical scene.

3. To understand the progress of a given investigator in a given year, one has to know the background and context of his work and be prepared to discuss his research progress in highly technical and scientific language, rather than in the general broad context of the science writer. ("High resolution" vs. "low resolution" seem apt terms to describe the two different levels of comprehension.)

4. In preparing the annual report, one is faced with the necessity of deciding whether the reports are to be couched in general lay language or in more accurate, highly technical terms. Usually, only an unsatisfactory compromise results, somewhere between the two.

Rather than attempting to cite a large number of events relating to small but significant steps in the attack on a broad range of problems in cell structure and function, we shall focus on several key areas where significant progress has been made or appears imminent.

Role of Microtubules in the Determination of Length

Dr. Joel Rosenbaum of Yale (GM 14642) has been conducting a fascinating series of experiments on the role of microtubules in the determination of length in cell processes. In experiments on flagella, Rosenbaum has found the flagellated protozoan Chlamydomonas a valuable experimental organism since (a) flagella can be harvested readily in large amount without injury to the organism, which then regenerates new flagella; (b) mutant strains with paralyzed flagella make possible the application of highly sophisticated microbiological and genetic techniques to study the formation and function of microtubules in flagella.

Among their findings are:

-- The cell regulates its flagellar protein precursor pool according to demands made upon it by flagellar growth, and this pool can then be used for assembly of a complete flagellum without concurrent protein synthesis.

-- Flagella do not have a self-contained protein synthetic system and flagellar elongation occurs by assembly of preformed microtubule subunits at the tip of the organelle. The mechanism by which the preformed subunits are transferred to the tip is unknown.

-- Rosenbaum has demonstrated that flagellar microtubules are made up of two electrophoretically distinct proteins called tubulins.

-- Flagellar microtubule proteins are quite stable and turn over slowly as compared to the microtubules of nerve axons and the mitotic apparatus.

-- Although flagellar microtubules are quite stable, if one cuts off one of the two flagella of Chlamydomonas, the intact flagellum is resorbed while the amputated flagella lengthens, and when they both reach the same length they grow out together, showing that one flagellum regulates the size and rate of growth of the other.

-- A structure has been found at the base of mature flagella which connects the flagellar microtubules to the flagellar membrane. This structure is absent during flagellar regeneration, thus permitting the flow of cytoplasm up the shaft of the flagellum. It is quite possible this structure acts as a gate, permitting the flow of precursors to the tip, and closing off to stop growth. The discovery of the mechanisms of control of flagellar growth has become a major goal of this research.

Recently, Rosenbaum and his coworkers have extended their research on microtubules to the growth of neuroblastoma cells in tissue culture:

-- When grown in spinner flasks the neuroblastoma cells remain rounded up with no extensions or processes.

-- When allowed to settle and adhere to glass, they send out long processes called neurites.

-- When cycloheximide is added to the medium, stopping protein synthesis, very short neurites are formed. When colchicine is added, no neurites are formed. Thus, neurite formation requires (a) that the cells adhere to a surface and (b) that microtubules be formed to enable the neurites to extend and grow. When the cells are removed from their substrate, they withdraw their neurites, round up, and divide.

-- When cells are placed on cellulose acetate, they cannot adhere; if the cellulose acetate is streaked with palladium, they send out single neurites, thus enabling the controlled study of neuronal growth.

-- If microtubular extension is necessary for the growth of nerves, and if growth of nerves is essential for the growth of limbs, then regulation and control of growth of microtubules in nerves may be important for limb growth and regeneration.

Several important questions arise from this research:

-- In the growth of flagellar microtubules, do the precursor subunits flow through the microtubule or in the space between the flagella and the microtubule?

-- In nerves, do microtubules run the full length of a nerve axon to serve as a passageway for precursor subunits, or for hormones; or is a given microtubule much shorter than the nerve axon, serving only as a structural element to provide rigidity and form to the nerve?

-- In the formation of neural processes, what is the significance of adherence of the cell to a surface? Does a surface phenomenon give direction and trigger the growth of neural elements? Recent evidence presented by William Kirkland (an NIAID grantee) and Paul Burton, an NIGMS career development awardee (1-K3-GM8620) suggests that cyclic AMP may have a role in regulating the formation of microtubules and, if so, might possibly be the mediator for the surface phenomenon.

Structural Basis of Sperm Motility

Dr. Ian Gibbons of the University of Hawaii, supported for five years under GM 15090, has become a leader in research on flagellary and ciliary motility. His recent application for continuation support for a project entitled "Structural Basis of Spermatozoan Motility" has been assigned to and accepted by the NICHD. Although a loss to the program of the NIGMS, this movement of basic scientists from research initiated in a very fundamental area, to an applied problem in one of the categorical Institutes--as basic knowledge reaches a point where it can be applied--is a rather frequent occurrence among NIGMS grantees. Thus NIGMS will often train an investigator, give him a base of support for initiating an important research area, and then within a few years he may move on to a more applied program of one of the categorical Institutes.

Because of the importance of Dr. Gibbons' work on GM 15090 during the period 7/1/67 to 7/1/72, it will be briefly summarized here:

-- The axoneme of sea urchin sperm flagellum consists of a cylindrical bundle of nine outer pairs of "doublet" tubules surrounding two single central tubules.

-- A double row of projections or "arms" runs along the "A" tubule of each outer doublet, extending toward the "B" tubules of the outer doublet. The proteins of these tubules, called tubulins, have slightly different properties and have a molecular weight of about 55,000.

-- A protein called dynein has been located in the arms of the outer tubules; this protein has been shown by Gibbons to have Mg^{++} activated ATPase activity.

-- The A and B tubules of each doublet are also connected by a set of "nexin" links that run from one doublet to the next. This protein has been isolated and appears to have a molecular weight of about 165,000.

-- Two theoretical models have been developed for the mechanism of flagellar movement: (a) a local contraction model involving localized shortening of the elements on one side of the flagellum or the other, and (b) a sliding filament model involving active sliding of the tubular elements.

-- The work of Gibbons supports the sliding filament model. Evidence gathered suggests that in the presence of ATP the dynein arms have a mechanochemical role in generating the force that leads to sliding between the tubules.

-- If the sea urchin sperm are extracted briefly with 0.5 M KCl in the presence of Triton X-100 and then transferred to a solution containing 1 mM ATP, the flagellary beat of 15 beats/second is about half that of control sperm, treated with Triton X-100 in the absence of 0.5 M KCl, which beat at 31 beats/second. Examination by electron microscopy shows that extractions with 0.5 M KCl has removed the outer dynein arms from the doublet tubules, leaving the inner arms intact. By varying the duration of KCl extraction, it could be shown that the rate of decrease in beat frequency paralleled the loss of the outer row of dynein arms. These results indicate that the rate of sliding between tubules is proportional to the number of dynein arms present.

-- Digestion of the axonemes of sea urchin sperm with trypsin modifies the structure so as to make it highly sensitive to ATP. Addition of ATP causes rapid disintegration into individual doublet tubules. Films taken of these doublet tubules show the doublet pairs sliding at different speeds in the same directions and in some cases in opposite directions.

-- Partial demembranization of sea urchin sperm by solution containing KCl, $MgSO_4$, EDTA, dithiothreitol, and Tris HCl buffer at pH 8.0 results in a suspension of sperm in which proximal, medial, or distal portions of the flagellum were membrane covered, while the remaining portions were naked. Treatment of these partially demembranated flagella with ATP caused the naked portions to become

motile while the membranous portions remained stationary. On the other hand, treatment with ADP caused the membranous portions to become motile while the naked portions showed little or no movement--this presumably resulting from the localized formation of ATP by adenylate kinase in the membranous covered portions. These results indicate that any region of the axoneme has the capacity for autonomous bending and that the beat frequency of a given region is determined by the local ATP concentration in that region.

-- The above work of sperm motility was carried out on sea urchin sperm. The structure of mammalian sperm is somewhat more complex, with an outer row of thick peripheral fibers and an outer fibrous sheath. Gibbons plans to study the mode of action of mammalian sperm in the coming year. His continuation grant was transferred to NICHD under HD 06565-06.

Formation, Structure, and Function of the Mitotic Apparatus

The NIGMS supports a number of investigators working on problems related to cell division:

-- Dr. T. C. Hsu and coworkers, of the University of Texas in Houston (GM 15887-05) have carried out a quantitative analysis of the mitotic spindle during cell division. They find (a) that the number of microtubules in the spindle of a given species remains approximately constant from cell to cell; (b) during spindle elongation the total number of microtubules remains approximately constant, but the chromosomal microtubules shorten whereas the continuous microtubules, extending from one centrosome to the other, increase in length. No evidence for sliding of microtubules was observed.

-- Dr. Paul R. Gross of the University of Rochester (GM 13560) finds (a) that the sea urchin egg has a pool of colchicine-binding proteins the size of which does not change during development; (b) the pool is drawn on continuously for formation of both temporary structures (the microtubules of the mitotic apparatus) and permanent structures (the microtubules of cilia); (c) the size of the pool is maintained by continuous synthesis of tubulins; and (d) the messenger RNA encoding the tubulin message is maternal in origin, since it is present and translated at normal rates even if synthesis of new RNA is blocked after fertilization.

-- Dr. Robert Kane, University of Hawaii (GM 14363) finds (a) that tubulin from the mitotic apparatus, sperm tails, and cilia appear to be identical upon analysis by ouchterlony immunodiffusion techniques; (b) the 4-5 S tubulins of the mitotic apparatus can be separated from a larger 22S "matrix" protein of 880,000 molecular weight by ultracentrifugation; (c) a third protein, called hyaline, has been isolated which is associated with the cortical granules of echinoderm eggs and is released at fertilization and acts to hold the blastomeres together during early development.

-- Dr. Daniel Mazia, University of California, Berkeley (GM 13882) finds that (a) when Na^+ in the fluid surrounding sea urchin eggs is replaced by Li^+ the mitotic apparatus is formed normally but breakdown is prevented; microtubules appear to be formed and disappear normally, but some other component is stabilized and does not break down; (b) the use of Li^+ permits recovery of the apparatus in high yield for biochemical study; and (c) a Ca^{++} activated ATPase has been recovered from the isolated mitotic apparatus.

-- Dr. Patricia Harris of Oregon State University (GM 19462) has just begun a study of a system of vesicles always associated with the mitotic apparatus but heretofore generally ignored. Their possible role in intracellular ionic regulation will be investigated using isolated vesicles and $^{45}Ca^{++}$.

New Developments in Electron Microscopy

Over the years, the NIGMS has made considerable contributions to the development of electron microscopy. About seven years ago, the Institute sponsored a two-day workshop on the possibility of developing high-resolution, atomic scale electron microscopy. Twelve leading electron physicists, engineers, and microscopists reviewed the state of the art, and identified promising areas for further development. Fortunately, this conference was well timed. Many useful, technical developments had emerged from the space research program: ultra high vacuum technology, low vapor pressure materials, vibrationless vacuum pumps, cryogenic techniques, superconducting materials, new magnetic alloys, image processing techniques, etc. These materials and techniques were discussed at length and recommended for further refinement and incorporation into a new generation of electron microscopes (EM). The basic designs of commercial EM had been developed about 40 years ago, and the conferees agreed that until new approaches were used, there was little possibility of any further major improvements for high resolution EM operation.

The NIGMS Advisory Council in March 1966 warmly endorsed the recommendations of this workshop, and Institute staff have since been participating in the efforts of several informal groups interested in developing electron microscopy in this country. These include a high voltage group under Dr. Robert Fisher of U.S. Steel, and a scanning electron microscopy group under Dr. Thomas Everhart at the University of California, Berkeley.

Several university research laboratories involved in the development of electron microscopes are supported by NIGMS funds. Current support is in the area of $1.4 million. The primary goal of these NIGMS-supported investigators is the development of instruments for high resolution electron microscopy approaching the 1 Å scale for base sequence identification in nucleic acids. Key projects in high resolution electron microscopy development are being conducted at the University of Chicago (Dr. Elmer Zeitler, GM 18391), Cornell University (Dr. Benjamin Siegel, GM 16195), Johns Hopkins (Dr. Michael Beer, GM 08968), and Oak Ridge National Laboratory (Dr. Ted Welton, Interagency Agreement 40-172-69). This is a multifaceted program among which the strategies are:

-- to increase the voltage up to the 1-million electron volt (mev) level to increase resolution and reduce beam damage to the specimen. Several high voltage power supplies have been constructed and are being tested.

-- to improve the design and operating characteristics of electromagnetic and electrostatic objectives for the electron microscope to reduce spherical aberration, which is a principal factor limiting resolution.

-- to use computer image processing techniques to increase the amount of information obtainable from the electron microscope.

-- to apply scanning techniques to isolate smaller portions of the sample for examination at any given instant so as to minimize scattering and increase the signal to noise ratio.

-- to use alternate imaging modes, such as fluorescence and electron absorption spectroscopy for obtaining different types of information from the specimen.

-- to operate at liquid helium temperatures (4°K) to minimize atomic motion and to lessen the effects of bond breakage from the electron beam.

Recently efforts to utilize very high voltage instruments (well above 1 mev) for study of cell organelles at 100 Å resolution have been undertaken by the DRR as a complement to the NIGMS program. This is closely coordinated with the NIGMS effort.

The NIGMS has played a similar role in the development of the scanning electron microscope (SEM). The SEM progressively passes a finely focused beam, raster-fashion, over each image element of the specimen. The scanning principle has been known since 1920, but it was not until 1960 that electronic techniques became sufficiently advanced to permit a sophisticated application to electron microscopy. Apparently the first SEM's in this country were built at the research laboratories of IBM (1961) and Westinghouse (1962) and used for materials research. Simultaneously (1961) Dr. Albert Crewe, now at the University of Chicago, with Dr. Zeitler, started an SEM development program at Argonne National Laboratory. The first university-based instrument was constructed by Dr. Tom Everhart at Berkeley in 1964 for semi-conductor and transistor research. Everhart soon realized that there were important applications of scanning electron microscopy in biological research and applied for and received NIGMS support beginning in 1967. His program has had three objectives:

-- to develop new instrumentation to improve the resolution and information output of the scanning electron microscope;

-- to develop new biomedical applications of the SEM;

-- to help other biological scientists determine how the SEM can aid in their research.

Technical developments include the development of a field-emission gun, a computer-controlled SEM, a transmission SEM, and a cryogenic SEM.

In the biomedical application of scanning electron microscopy, this group has been particularly interested in studying the architecture of the central nervous system. For several years they have sought methods whereby they could satisfactorily expose the surfaces of nerve cells in order to study the neuronal architecture and the organization of neural circuitry. Selective digestion with enzymes proved reasonably successful in certain invertebrate preparations and in peripheral sensory structures of vertebrates. However with tissue from the vertebrate central nervous system, the problem involves removal of supporting glial cells and interstitial material. The technique finally developed involves silver precipitates around the desired structures and selective removal of the organic material in a low-temperature oxygen plasma generated at low pressures by a radio-frequency electric field. Working carefully to avoid artifacts, they have been able to clearly discern neuronal structures not easily, or at all, resolvable by light microscopy. The method is being checked on preparations of human cerebellar cortex where neuronal architecture and organization are well known in order to detect sources of possible artifacts. Also being studied are vestibular system receptors and a new kind of invertebrate ciliated chemoreceptor. In the future, extensive work is planned on the Golgi apparatus and other cellular organelles.

In related developments, John M. Cowley of Arizona State University (GM 18204) is working toward the development of a high-voltage scanning transmission electron microscope (STEM) for the observation of hydrated and, perhaps, even living specimens maintained in air at atmospheric pressure ("alfresco" microscopy). This instrument, which is nearing completion, should permit the observation of cells and microorganisms in their natural state, and perhaps even permit study of the behavior of living motile organisms.

Whereas the standard electron microscope operates in the 100 kev (kilo electron-volt) range, and high-voltage electron microscopes are now being built in the 1-10 mev (million electron-volt) range, Dr. Alvar Wilska of the University of Arizona is developing a low-voltage electron microscope in the 2-6 kev range with a resolving power of about 5 Å. The use of low voltage provides greater contrast and should provide new information on the primary, tertiary, and quaternary structure of viruses, nucleoproteins, nucleic acids, and proteins. These techniques, in combination with the specific base staining techniques developed by Beer, may make possible the determination of the base sequence of DNA. Wilska has done more than any other investigator in the world to develop low-voltage electron microscopy as a practical technique.

Epilogue

The present annual report has surveyed briefly the scientific and administrative events which led to the formation of a program within the NIGMS on the cellular basis of disease and various considerations relating to the organization and structure of the program. The research highlights have necessarily been brief and have reviewed progress in several areas related to research on the whole cell and cellular organelles; namely, the role of the microtubules in the determination of length; the structural basis of sperm motility; the formation, structure, and function of the mitotic apparatus; and new developments in electron microscopy. The large bulk of the program dealing with biophysics and biochemistry are not covered in this review and will form the basis of subsequent annual reports. Two subjects, (a) research on membranes, and (b) changes in the conformation of proteins in relation to their function, appear to be prime topics for consideration in the next report.

CLINICAL SCIENCES PROGRAM

I. Program Content

A. Research

This year the Clinical Sciences Program has shown a marked improvement in the depth and substance of its research. It has focused its attention on basic research as a foundation upon which to build a strong program. Such emphasis should provide more impact to clinical application in the future.

This has also been a time for re-appraisal in the program. The three clearly defined areas (Anesthesiology, Diagnostic Radiology and Trauma) have been evaluated as to past accomplishments and more carefully considered as to their future needs. Several major accomplishments have had direct application of research to patient care. Patient monitoring, formerly available only to research beds, is now recognized as a service need for all seriously ill patients in Parkland Hospital, Dallas. Specific advances have permitted recognition of the effects of certain anesthetics and drugs in accentuating neonatal hypoxia during birth; rapid evaluation of the comprehensive effects of new anesthetic agents, e.g. ethrane; accurate fluid balance during resuscitation; monitoring of blood gas concentrations after injury; the use of end expiratory pressure ventilation and diuretics to prevent the life-threatening pulmonary insufficiency following trauma; and early diagnosis and prevention of post-burn sepsis. Through the combined talents of clinicians, scientists and engineers, laboratory research devices such as blood gas monitors, pH meters and spirometers have been put to widespread clinical use. A critical look at each of the components revealed definite weaknesses as well as strengths within the program.

Trauma: In trauma it became apparent that emphasis must be placed on the individual research project with in-depth study of one aspect. Almost every accident results in a chain of events or series of interactions. It is not practical or possible to study each factor separately, yet certain aspects must be studied in-depth before they can be assessed as part of the whole complex picture of trauma. For instance, it is important to follow through on biochemical and electron microscopy studies which have demonstrated abnormalities in mitochondrial respiration, oxidative phosphorylation, and intracellular energy transfer. Lysosomal disruption and intracellular scatter of acid phosphatase which occur rapidly in intestinal epithelium after severe hemorrhagic hypotension need further evaluation. The turnover of glycoprotein fractions altered by stress has been related to humoral substances which act upon glycoproteins during shock. What is the significance of these changes? Such new research projects have become almost non-existent in the last few years, despite the fact that trauma continues to be the number one killer of children and young adults, and the mechanisms involved have yet to be fully understood.

Assessment of the on-going trauma projects which were activated when the program first started have revealed major break-throughs in this complex field and have shown what can happen when neglected areas are cultivated. These investigations have led to the establishment of specific

therapeutic guidelines for such life-threatening processes as irreversible shock, sepsis following burns, post-traumatic pulmonary distress syndrome, etc. There are still many unexplored avenues of approach to this serious health problem, and more such projects are needed. Therefore, special staff emphasis on these projects led to the submission of a greater number of trauma-related applications during the year. As a result, the number of projects considered worthy of support by this Institute is triple those of recent years.

Anesthesiology: An assessment of the Anesthesiology program revealed significant examples in which a full range of investigations from the most applied to the most basic types could be found. Although the NIGMS in the past had played a major role in fostering and preserving the scientific basis for this field, it has recently had little opportunity to nurture new research. This was due in part to a paucity of new applications, but also resulted from a tendency for the research to be categorized into disease areas and thus fall within the purview of another Institute. Much of the original research came up for renewal this year, thus providing an excellent opportunity to assess its current merit. It was clearly evident that these research efforts were the building blocks for significant improvements in total patient care by the anesthesiologist. The application of his clinical and research skills have led to great advances in modern surgery and intensive care medicine. Today, for example, a patient placed on a respirator in the intensive care unit of any major medical center has an 85-90 percent chance of survival as compared to 25 percent ten years ago. Basic research on diffusion of anesthetics using multiple radioisotopes and mathematical analysis of transit times by computer has led to a generally useful method for the study of capillary exchange.

Much of the past research efforts in these renewal applications on anesthesiology was considered of high quality and protocols for future work were promising. However, there were certain weaknesses which appeared to need further attention or even deletion to assure continued accomplishments in this field. These include the lack of innovative protocols on the structure-activity studies and the absence of comprehensive laboratory investigations of new anesthetic agents before trials in human subjects. Of major concern was the relative lack of active participation by the principal investigators in both research and administration. It was felt that these weaknesses were more clearly understood and corrections made easier as a result of better communication and interchange of ideas during and after these critical reviews.

Diagnostic Radiology: During the year, Diagnostic Radiology has broadened its scope of research to help meet the demands placed on the radiologist by the many technological advances of modern medicine. The radiologist functions primarily in a hospital setting where his service is required by every specialty. Much time is devoted to studies of the diagnostic radiologic examination itself in terms of more accurate film interpretation, radiologic therapy and biologic effect. There is a constant need for the development of improved diagnostic tools and adjunct computer systems. Such research is currently supported by the NIGMS and includes studies involving ionizing and infrared radiation, thermography, radioactive isotopes, and ultrasonic techniques for investigating structure and function in disease.

Diagnostic nuclear medicine, which involves the preparation and use of radioactive materials in medical diagnosis, has become increasingly important and lends itself to research in laboratory techniques, dynamic function studies, regional blood flow studies, and organ visualization. The value of nuclear medicine is being demonstrated in the study of specific organ function after trauma, and in evaluating techniques of organ preservation, to name only a few examples. Studies of the preparation and use of isotopes should be encouraged and will play an increasingly important role in providing sound methodology for programs of vital clinical importance.

Centers: In FY 72 the clinical sciences program was expanded by the addition of three new research centers (one in trauma and two in diagnostic radiology). Also four of the on-going centers and program-projects were approved for renewal and expansion of their research (three in anesthesiology and one in trauma). The diagnostic radiology centers were the first activated by the NIGMS. The Institute has had a special congressional mandate for these centers through enactment of the Heart Disease, Cancer and Stroke Amendments of 1965; however, these two research centers were the first deemed worthy of such support in this area. The centers are located at Harvard University and at the University of Chicago Medical Center; both have developed from solid on-going research projects by experienced investigators. At the Harvard Center, under the direction of Dr. Herbert L. Abrams, new angiographic and isotopic techniques are being developed, and correlative information is available through close cooperation with members of the Departments of Medicine and Physiology. Of particular interest and importance are models of acute renal failure and of mitral insufficiency being developed in dogs which will hopefully lead to greater understanding of these diseases.

At the University of Chicago, Dr. Kurt Rossmann is developing a program in nuclear medical images and image systems. Emphasis is placed on relating the properties of x-ray and radio-nuclide images to their diagnostic utility.

It is recognized that there is a great need to establish rapid lines of communication from the engineers and physical scientists evaluating and designing these diagnostic tools to the clinicians who use them. This is highly emphasized in both of these new centers and provides, at least in part, the basis for their support.

The new trauma research center is located at the University of California, San Francisco, under the direction of Dr. William Blaisdell and involves a multidisciplinary effort on the part of the Departments of General Surgery, Neurosurgery, Anesthesiology, Orthopedics, and Pathology to promote basic and clinical research on such critical problems as microcirculation and coagulation following traumatic organ system failures. This center, the first trauma center on the west coast, is also the outgrowth of several solid on-going research projects supported by the NIGMS.

In addition to the specific targeted areas of the program, the Institute supports a small number of clinically revelant, non-categorical projects in the areas of general surgery, basic pathology, tissue preservation and epidemiology.

B. Manpower

In the clinical sciences program of the Institute, training grants have been awarded to medical schools to increase the numbers of research scientists within the very active clinical disciplines of anesthesiology, diagnostic radiology, and surgery. Although there is indication that the critical shortage of professional personnel is lessening, the shortage of trained academic manpower is still acute and the numbers of research scientists in these disciplines are relatively few.

Anesthesiology: Anesthesiology has developed as a significant specialty in the past two decades. Its scientific base has grown in depth and breadth with the NIGMS research training grants program playing a significant role in the improvement of the manpower supply. The products of this program, however, provide only about one-third of the medical school faculty requirements in anesthesia. A review of 252 individuals who completed NIGMS-supported research training shows: 139 (55%) now in academic positions, 80 (31%) in non-academic work, 32 (13%) in the military, and one deceased. During 1971-72, 12 programs were awarded ($896,114); two predoctoral and 68 postdoctoral trainees were supported.

The research activities of anesthesiology trainees reveal a broad spectrum of interest. Clinical applications include: reduction in mortality and morbidity in intensive care units, prevention of drug-induced cardiac arrest during anesthesia, better management of intra-operative and post-operative bleeding problems, and more rational treatment for millions of patients with cerebral vascular problems.

The average length of training in anesthesiology has been 18 months. It has become evident to staff and consultants alike that trainees in academic anesthesiology should be required to spend a minimum of two years of concentrated study in basic science and research. Short of this, they would not be adequately prepared for academic careers. The Program Directors are now beginning to move in this direction.

Within the regular fellowships program, anesthesiology was represented by two special fellowship awards in FY 72 as compared to one for last year. Both of these special fellows will be working under the supervision of investigators in anesthesiology research centers supported by the NIGMS.

The number of research career development awardees in anesthesiology dropped from seven in 1971 to five in 1972. Despite a gradual decline in the number of awards since 1970, the NIGMS proportion of all NIH awardees remained constant from FY 67 to FY 71. During these years, the NIGMS supported 80 percent of all NIH awards to anesthesiologists. Since that time, all Development Award applications received from anesthesiologists have been assigned to the NHLI, and for the first time in five years the number of Awardees supported by the NHLI will exceed the number supported by the NIGMS.

Clinical Anesthesiology: On June 30, 1972, the 29 clinical anesthesiology training grants supported by the NIGMS were phased out. It was felt that

if the Institute is to have a future role in clinical training, its emphasis should be on the improvement of quality rather than simply adding to the number of practicing physicians in anesthesiology or any other specialty.

Radiology: The radiology research training program of the Institute is relatively new and has only recently begun to provide trained manpower for academic radiology. Unfortunately, enough trained competent investigators simply do not exist in radiology to support the large expansion of research efforts needed, and a large infusion of research funds without associated training support would not solve the problem. At this time, there is a need to provide small grants for the initial funding of the research of trainees who have just recently completed their training. Another problem is the lack of breadth of research by radiologists, who tend to limit their efforts to the investigative modalities which are available to practicing clinical radiologists. Closer liaison with basic science disciplines and more opportunity and encouragement to take course work in research methodology, statistics, experimental design, advanced mathematics, and in the basic sciences should help to overcome this difficulty.

Important research progress has been made in developing accurate methods for computer processing of radiological data and has led to more rapid retrieval of information by clinicians. Basic research on radiological imaging systems has also resulted in improvements in equipment and technique and has greatly reduced the hazards of overexposure of patients to radiation.

The funds awarded have been relatively constant at approximately $1.8 million, increasing by $47,000 during the current year. No new grants were awarded during the past year. Twenty-three grants (14 in diagnostic radiology and nine in nuclear medicine) were active, supporting 124 postdoctoral trainees and two predoctoral physics students. Three diagnostic radiology training grants terminated June 30, 1972.

Through June 30, 1971, a total of 221 trainees had been appointed for periods longer than six months, of whom 64 are now in academic positions, 35 are in non-academic work, 24 are in the military, two are unknown, one is deceased, and 95 are still in training.

Within the fellowships program seven postdoctoral and special fellowship awards and one research developmental award were made. This is an increase of five over last year. Dr. David Shames, Department of Radiology, University of California, San Francisco Medical Center, represents the first diagnostic radiologist recipient of an NIGMS Research Career Development Award.

Surgery: From the surgery research training program there is emerging a cadre of well-educated clinical scientists with an effective combination of basic science and clinical competence. The goal of the training is development of a surgeon, who not only delivers excellent patient care but also is sufficiently curious to seek and recognize through laboratory and clinical investigation the solution to problems in human biology as related to surgery. Surgical scientists have made significant contributions in gastrointestinal physiology, coagulation, biochemistry, and immunology. A significant portion of the trainee's program involves study in basic science with opportunity

provided for research during the clinical training years.

In the funding of surgery training grants, there has been a decline from the maximum of 32 programs supported in 1969-1970, to 30 programs active in 1971-1972. Five additional training programs will terminate June 30, 1972. Of the 30 programs, nine programs were reviewed for continued support beginning July 1, 1973. One of these was disapproved. Unless additional awards are made, the number of active programs will total 24 by the end of June 1973.

From the beginning of this program to June 30, 1971, 294 trainees have been supported for a period of six months or more. Of these, 79 are now in academic positions, 32 are in non-academic work, 30 are in the military, the positions of six are unknown, one is deceased, and 146 are still in training. Although this is a postdoctoral program, trainees may earn advanced degrees. To date, 12 Ph.D. degrees have been awarded in physiology, microbiology, biophysics, biomedical engineering, immunology, and surgery. One M.S. degree has been awarded in surgery and physiological chemistry. One hundred and thirty postdoctoral trainees were appointed during the past year.

Future directions for this program include an increasing emphasis on the multifaceted problem of trauma. We hope to direct this interest not only to a basic understanding of the mechanisms of injury and repair, but also to improved methods of care for the trauma victim including initial treatment and prompt evacuation, definitive therapy at appropriate centers, and adequate rehabilitation. To this end, closer liaison between trauma research centers and surgery training grants is being encouraged and increasing emphasis will be placed on programs offering significant improvements in the care of the critically ill.

The following five schools which have Trauma Research Centers funded by NIGMS, also have surgery research training grants in the academic surgery training program:

*Columbia Presbyterian Medical Center
New York, New York
Focus: Studies of human energy exchange and changes in body metabolism following severe trauma

Hospital of the University of Pennsylvania
Philadelphia, Pennsylvania
Focus: Shock at the cellular level

*University of Texas Southwestern Medical School
Dallas, Texas
Focus: Comprehensive baseline studies on the traumatized patient

*University Hospital, Boston University Medical Center
Boston, Massachusetts
Focus: Correlation of pulmonary function with hormone metabolism and intracellular chemistry in trauma and shock

San Francisco General Hospital
University of California, San Francisco
San Francisco, California
Focus: A multidisciplinary approach to trauma combining clinical and animal studies in the areas of wound healing, coagulation and microcirculation changes, and pulmonary changes following positive pressure ventilation

*It is noteworthy that three of the principal investigators of trauma centers are program directors of academic surgery training grants as well. In 1971-1972, approximately 30 trainees were appointed to training grants in these five schools.

Special fellowships in the area of trauma dropped from 14 awards last year to only five in FY 72, and of these two were new awards. Eleven fellows presumably completed their training in FY 71. RCDA support was continued for Dr. Leena Mela, Department of Surgery, at the University of Pennsylvania, whose work in trauma was first supported by this Institute in FY 71. The small number of NIGMS Awardees in diagnostic radiology and trauma reflects a virtual absence of applications in these areas up to the present time. However, applications for RCDA's in both areas are scheduled for review during FY 73. Thus, it is anticipated that the number of Awardees in diagnostic radiology and trauma may double during the coming year.

Staff has been active in stimulating an assessment of the continued needs for research-trained manpower. On September 10, 1971, a meeting of the surgery training program directors was held in Bethesda, Maryland, to review the future status of surgery training grants and the appropriate role they play in the training of academic manpower. The participants identified the need for small research grants, available to recent graduates of the training program, before they have had time to establish themselves and apply for support through the usual channels. It was noted that while there was no shortage nationally of physicians doing surgery, board qualified surgeons were still in short supply and academic surgeons especially so. A factor which tends to increase the demand for academic surgeons is the relatively short period that a surgeon scientist remains creative and productive in the laboratory. After 10-15 years, the majority become laden with administrative responsibilities. The academic needs for surgical manpower and research will be further evaluated by a comprehensive study ("SOSSUS Evaluation of Surgical Research" contract) initiated at the end of FY 72.

As an outgrowth of the Program Directors' meeting, the Surgery Training Committee and the NAGMS Council recommended minor revisions in the Guidelines, initially written in February 1966, to conform to policy and program changes which have taken place since that time.

A meeting of the Program Directors of the diagnostic radiology training grants program was held January 17-18, 1972. The evaluations were made of the existing programs as to accomplishments, trends and future needs. It was recommended that the program be not only continued but expanded to

achieve the goal of adequate staffing for all departments of radiology in the nation's medical schools.

"Research Training in Radiology," a report written by the Radiology Training Committee in June 1971, was published by the Government Printing Office during the current year. Approximately 2,000 copies were distributed to interested persons. It is believed that this is a reflection of the current interest in manpower in this discipline.

The following chart shows training and fellowships awards for FY 72 that directly relate to the existing research centers and program projects.

Area	Research P.P & Centers Grants	Training Grants	Fellowships	Research Career Awards
Anesthesiology	7	7	2	3
Diagnostic Radiology	5	4	1	1
Trauma	10	5	4	1

C. Research Highlights

J. Bunker, Stanford University School of Medicine, Stanford, California, GM 12527

"Teratogenicity of Anesthetics"

Demonstration that inhalation anesthetics produce teratogenetic responses in both avian and mammalian species has raised concern as to their effects in an estimated 50,000 women in the United States who annually undergo surgery and anesthesia during gestation. Dr. Bunker and his team of investigators have recognized that a second group of women are also exposed to the effects of inhalation anesthetics. These are the nurses and anesthetists who work daily in operating rooms, contaminated with trace concentrations of these gases. Studies recently completed at Stanford University confirm a contamination of the operating rooms with waste anesthetic gases, and indicate an increased spontaneous miscarriage rate in operating room nurses and female anesthetists which is 2.9 and 3.7 times the control group. Also, it was shown that an 85-90 percent reduction in the concentration of waste anesthetic gases found in the operating room atmosphere can be achieved through the use of appropriate scavenging devices. Such devices attached to the exhaust valve of the anesthesia machine can readily be installed in most operating rooms at nominal cost. These devices are being used in many of the hospital operating rooms and delivery suites throughout the country.

J. Bonica, University of Washington Medical Center, Seattle, Washington, GM 15991

"Pain: Where Does It Hurt?"

"Pain: Where Does It Hurt?" is an NBC hour long documentary presented to TV audiences in 1972. It received the greatest public response of any documentary in the history of NBC. On the basis of the NIGMS brochure, Pain, and the Institute's film, Threshold, the documentary was developed around Dr. Bonica's pain center. The research involving the patients in this center is supported under the anesthesiology research center grant. This research involves (1) developing more precise measurements of the pain experience in both the clinical context and the laboratory and (2) evaluating the effects of morphine, aspirin, placebos, and hypnosis on pain. This group of investigators is currently studying the effects of diazepam on the tolerance of tourniquet pain and state of anxiety during pain. The drug and a matching placebo are administered in two separate experimental sessions. State of anxiety during time in pain increases over time when subjects have taken the placebo but decreases substantially over time in pain when subjects have taken diazepam. Diazepam leads to significantly greater pain tolerance times than does the placebo.

W. K. Hamilton, University of California, San Francisco, California, GM 15771

"Evaluation of New Anesthetic Agent, Forane"

Dr. Eger, one of the investigators in this program-project on anesthesiology, has been evaluating the new agent, Forane.

Currently used anesthetic agents have a remarkable record of safety when the degree of drug depression necessary to produce general anesthesia for surgical procedures is considered. However, there are inherent and seemingly unavoidable side effects which cause a continuing search for new and better agents with which to provide anesthesia. A series of seven articles in the July 1971 issue of Anesthesiology report several of the initial studies of this team with the proposed new agent. The investigations have been carried out in laboratory animals and man and have now progressed to the stage of clinical trials. The new drug boasts several potential advantages, e.g., cardiac output is maintained at control levels, and the heart does not appear to be sensitized to epinephrine. Further, the drug does not appear to undergo metabolism in the body. Evaluation of this new agent is greatly enhanced by critical comparison with currently used anesthetic agents using techniques worked out by Dr. Eger under previous support by this grant. A good deal of further information must be gathered before the drug is declared available for regular operating room usage.

H. N. Wagner, Johns Hopkins University, Baltimore, Maryland, GM 10548

"Rapid Antibiotic Sensitivity Testing"

Dr. Wagner and his team of investigators, working in the radiology research program-project, have developed a new and accurate method for rapid, automatic radiometric testing of antibiotic sensitivity within three hours following inoculation of a culture medium. This is compared to the conventional tube dilution technique of antibiotic effects on bacterial growth which has taken 48-hours to complete in most laboratories. The radiometric method measures the amount of radioactive CO_2 generated by the bacterial metabolism of ^{14}C-glucose in the presence of antibiotics. Antibiotic effect on bacterial growth is standardized by measuring the evolution of $^{14}CO_2$ three hours after inoculation. The accuracy of the two methods run in parallel and therefore, the use of the radiometric method would permit early administration of the proper antibiotic in such life-threatening conditions as septicemia.

K. Rossmann, University of Chicago, Chicago, Illinois, GM 15806

"Improved Angiograms Make Cerebral Pathology More Detectable"

It has been shown from physical measurements and clinical radiographs that the sharpness of cerebral angiograms can be improved significantly by using a combination of slow intensifying screens and fast film rather than the commonly used combination of fast screens and film of normal speed. The resulting increased visibility of smaller blood vessels makes it feasible to study their diagnostic significance. No degrading effects due to poor screen-film contact and motion of the film changer were observed. Other methods of improved image processing are being continued in the new research center grant awarded to Dr. Rossmann in June, 1972.

R. H. Egdahl, Boston University Medical Center, Boston, Massachusetts, GM 17366

"Accurate and Rapid Correction of Hyperglycemia Following Shock"

Hyperglycemia is a common entity of the shock syndrome. Patients admitted to the hospital with severe burns or in hemorrhagic shock are routinely given insulin and glucose despite the fact that relatively few data are available to suggest exactly how this should be done. Dr. Egdahl and his associates have devised a method for directly measuring insulin secretion rate in man. This is of great importance in understanding the dynamics of the insulin system. Previous studies have been based on measurements of insulin concentrations in the peripheral blood. These are not considered true measurements since pancreatic insulin enters the portal venous system where it is altered by the liver secretions. The Boston University group has devised a technique in which cannulas are placed in the umbilical and portal veins permitting measurement of blood flow and insulin secretion. Using a continous infusion of lipiodol, it is possible to accurately measure portal blood flow; and this, multiplied by the portal insulin concentration, provides the means for determining insulin secretory rate. Currently this method is used to measure effects of epinephrine, corticosteroids, and other critical hormones on

insulin secretion during hemorrhagic shock. The investigators conclude that hyperglycemia results from epinephrine release, which inhibits insulin release from the pancreas. Another conclusion drawn is that the adrenal medullary secretions inhibit glucose assimilation in shock, irrespective of the effects of corticosteroids. Findings suggest the use of adrenergic blocking agents might be a rational approach to increasing glucose assimilation. Continued research in this area should provide information on which vital decisions regarding treatment can be made.

S. Powers, Albany Medical College, Albany, New York, GM 15426

"Electronics Help Prevent Shock Deaths"

About 70 victims of severe trauma have been saved from possible death at the Albany research center because of the collaborative efforts of physicians and computer scientists. They have found through the use of electronic equipment a way to predict the occurrence of post-traumatic pulmonary distress syndrome, the most frequent cause of death following physical injury. The electronic equipment in the one-bed trauma room was developed by biomedical engineers at nearby Rensselaer Polytechnic Institute and is used to measure a patient's vital processes. Through the use of high speed computer analysis the results are returned within minutes to the bedside physician, permitting him to apply life-saving measures. Although the initial cost for developing the trauma room was high, Dr. Powers feels it can now be duplicated at a fraction of this cost.

F. W. Blaisdell, University of California, San Francisco, California, GM 18470

"I.V. Phosphate Possibly Eliminates Need for "Fresh" Blood Transfusions"

Recent studies have suggested that fresh blood should be used to transfuse patients suffering from post-traumatic shock, in which there is an oxygen deficiency in the tissues. This is because blood stored in usual liquid preservative for over seven days will increase its capacity to hold onto oxygen, theoretically diminishing the red cell's ability to deliver oxygen to the tissues. At this new research center, Dr. Blaisdell and his team have been successful in restoring the red blood cell's ability to unload oxygen by infusing a simple inorganic phosphate solution. If further studies confirm these findings, it would permit the use of banked blood which is currently the only blood readily available for emergencies.

II. Program Administration

During FY 72, the Institute continued to develop high quality programs in each of the defined clinical science areas. It was evident that in order to have strong programs in trauma and anesthesiology the Institute should support more individual projects with basic research orientation. Although a larger number of trauma project proposals were received, relatively few of these were considered of adequately high scientific merit. Also, the categorical focus of these applications precluded primary assignment to this Institute. It became obvious that better lines of communication are needed within the NIH as well as with the scientific community. As a result, a new set of referral guidelines for trauma were prepared in the hope that they will

more clearly define trauma and its continued role in the program of the NIGMS.

The status of the anesthesiology program revealed very little change in the numbers and dollars of grant support from the previous year. No new applications for anesthesiology research centers or program-projects were received and individual projects were considered primarily on the basis of their categorical focus. It therefore appeared that the Institute should concentrate on some specific areas of anesthesiology as a discipline. Renewed interest occurred in the area of pain research, and support was afforded for a pain symposium. The area of acupuncture research was assigned to the Institute by the Director, NIH, and plans made for a two-day acupuncture meeting at NIH July 17-18, 1972. There is continued interest in the pharmacokinetics of anesthetic agents, in new instrumentation for inducing and monitoring anesthesia, in management of respiratory emergencies arising from inhalation therapy, and in the relationship of projects in anesthesia and diagnostic radiology with trauma research.

In diagnostic radiology, efforts were concentrated on the development of a research center which would be worthy of support. The fruits of these labors were the awards for the two research centers. Activation of new individual projects in diagnostic radiology has continued at a steady pace.

This was a year to put the center concept to test. With major support of large program-projects and centers in the clinical programs and with an appreciable number of these having renewals at this time, it was felt that an adequate appraisal could be made. Consequently, a more critical look was taken of the review mechanism as well as the applications being reviewed.

The General Medical Program-Project Committee devoted its October 1971 meeting to a discussion of research centers. The primary objective was to formulate opinions and advice which could be used by staff in developing better guidelines for the Committee and site visitors. It was the opinion of the Committee that the major shortcoming of centers and program-projects relate to the inadequate preparation of proposals for such large programs. Although a careful review compensates in part for this deficiency, poor research may be more difficult to identify. This problem solving can best be achieved by more careful scrutiny for scientific excellence plus adequacy of administrative and environmental aspects.

Suggestions to improve quality control included the use of NIH consultants in teams as monitors, the appointment by the center director of an extramural committee of peers which might or might not submit reports to NIH stating deficiencies and corrective actions, the use of a review group composed of senior scientists from the applicant's own institution, and designation of grant funds to support frequent reviews by scientists chosen by the Director. The notion that centers should be problem oriented (e.g., "Trauma") rather than discipline oriented (e.g., "Anesthesiology") was mentioned.

It was felt that attempts to coordinate all center research nationally to avoid duplication should be encouraged, and that requirements for fixed ratios of basic to clinical science projects were not realistic. Relationships of a center to the university, medical school, and hospital should

be "excellent" and "subservient," and these relationships, which form an important aspect of the proposal, should be explored in depth during the site visit. A center represents a kind of conglomerate of program-projects designed to study problems of broad scope through a team approach. Both program-projects and centers were designed to meet national health needs through training and research, creating high visibility, "forced" cooperation, and core facilities, which provide with economy a fertile environment for young scientists and trainees, who could become productive early in their careers. An important distinguishing feature of a center is the primary commitment of the component investigators. Thus, centers may differ from program-projects only in size, but not in duration, scope of research problem, etc. It was considered important that the center include educational and clinical functions, as well as research activities. A summary of these concepts is as follows:

	Grant	Program-Project	Center
Size	S	M	L
Cost	S	M	L
Scope of Problem	S	M	L
Duration of Support	3-5 yrs.	3-5 yrs.	3-5 yrs.
Clinical Responsibilities	-	±	+
Educational Responsibilities	-	±	+

The role of the center as a community or regional resource was emphasized. Centers should have some responsibility for the level of care in the nearby community, at least in serving as examples of what can be done.

At the January 3, 1972, meeting of the Scientific Directorate of the NIGMS, Dr. DeWitt Stetten presented for discussion a draft of what he considered to be the characteristics of centers to be supported by the Institute. These points are discussed more fully in the Director's Report. They constitute very helpful guidelines to those interested in developing centers and also in reviewing such applications.

During the past fiscal year, the Institute has concerned itself with stimulating further interest nationally in trauma research. While deaths from other major causes are steadily declining, death from trauma continues to rise. Until there is a better understanding of the body's total response to injury and the development of adequate therapeutic measures, this death rate is unlikely to be reduced.

The proposals by Representative Rogers, in Congressional Bill H.R. 12563, January 24, 1972, and Representative Mazzoli, H.R. 13582, March 8, 1972, for additional support to NIGMS in the area of trauma were welcomed by the Institute. Such research would allow greater expansion of existing studies and make possible the activation of new centers, program-projects and regular projects to investigate still unexplored avenues to this

complex problem. These congressional bills would also provide support for programs in emergency medical services.

On February 2, 1972, there was a joint meeting of the General Medical Program-Project Committee and the Anesthesiology Training Committee. The objectives of this meeting were to provide a comprehensive picture of the present research and training programs in anesthesiology, to evaluate the importance of anesthesia research centers on training, and to discuss the impact of research and research training on academic anesthesia. The needs for further research were definitely established, and it was felt that the center program should continue. Although the partnership between basic and applied science is not ideal, the necessary bridges are being built. One limitation on the number of research centers is simply the shortage of good research programs. With the successful completion of research training by more anesthesiologists, this shortage should be alleviated.

Plans for FY 73 include program directors' meetings for each of the special emphasis research programs. Through these forums, the Institute expects to garner information on special opportunities, problem areas, and future needs. Armed with such knowledge, administrators within the programs will be better equipped to cope with the many challenges which they will be encountering in the years ahead.

ENGINEERING IN BIOLOGY AND MEDICINE

I. Program Content

Introduction

The main objectives of this Program are (1) to support the effective introduction of the principles and practices of engineering science into fundamental and applied biomedical research, and (2) to support quality research training in the area. In selected instances, often arising from research supported by the Institute, prototype instrumentation and devices are being developed that will be useful in the improvement of medical care.

The Program has two main segments--one in biomedical engineering and one in the automation of clinical laboratories. Over the past several years both have continued to be vigorous and productive.

In July 1972, the Senate Appropriations Committee chose to note that this overall effort has matured into a Program of national significance for health. The Committee feels that in order to exploit further the growing relationships between the fields of engineering, biology, and medicine, there is a need over the next several years to initiate and develop a program of biomedical engineering centers. They recognized that in such a program new institutional forms and linkages must be evolved between medical and engineering schools having the requisite capabilities, and that the efforts in such centers should be variously balanced between basic and applied research.

They further indicated that these biomedical engineering centers might interface effectively on a regional basis with some of our Federal laboratories and be productive in much the same way that the Institute's research and development program for the automation of clinical laboratories has been with the Oak Ridge National Laboratory and the National Bureau of Standards to date. With respect to these activities the Committee noted the remarkable performance of the GeMSAEC fluid analyzer, developed through an interagency agreement with Oak Ridge National Laboratory scientists, which vastly improves the speed and accuracy of blood and urine testing. Several commercial versions of the instrument are now in use in laboratories throughout the country. The work with the National Bureau of Standards centering on the development of highly purified, nationally certified reference chemicals was cited as greatly needed to verify the accuracy of laboratory test results and to ensure the uniformity of chemicals used for the calibration of instruments among different laboratories. This project thus far has produced 16 chemicals as reference standards, all of them significant for the improvement of the reliability of laboratory tests that are widely used by physicians in the diagnosis and treatment of disease and illness.

Presently, overall support for the Engineering in Biology and Medicine Program is as follows: research $11.4 million; training $3.3 million; fellowships $1.2 million; and development $1.8 million. This $17.7 million investment represents approximately 11 percent of the Institute's overall research, training and development effort.

A. Biomedical Engineering

Research

In the past year emphasis has been placed on the application of engineering theory and practice to research on the clinical evaluation and management of critically ill patients through (1) the development of new systems for rapid data acquisition and display that involve new types of transducers and integrated electronic circuitry, and (2) the development and application of mathematical models of physiological control systems to predict more accurately and rapidly the clinical status of the acutely ill patient.

Work in these areas has progressed well and has led to significant contributions for the management of the seriously ill. With support from the Institute, transducers and computerized systems for data analysis have been developed and installed in the patient monitoring facility of the Childrens' Memorial Hospital in Chicago. Largely based on these developments, this hospital has recently been designated as the major resource in the Chicago area for the care of traumatized children.

Applications useful in medical care have been made as a result of research on mathematical modeling of physiological control systems. Based on a mathematical model of kidney function, a nomogram has been developed which permits the physician prescribing artificial kidney treatment, in cases of kidney failure, to design an individualized dialysis regime for each patient. This will result in more efficient utilization of artificial kidney machines.

It is anticipated that emphasis during the coming year will be placed in four major areas: (1) Communication and control--further studies on the application of engineering systems science for the modeling and understanding of physiological control mechanisms. (2) Data processing--as the instrumentation brought to bear on problems in biology and medicine becomes more and more sophisticated, interpretation of the data obtained will necessitate an increase in the use of digital computation. Moreover, data processing techniques will be more extensively applied in the computerized analysis of such widely used methodologies in diagnostic medicine as reading of x-rays and the counting of blood cells. (3) Instrumentation--an era is being entered in the development and utilization of microelectronic devices for medical purposes, such as telemetering of physiological phenomena, control of prosthetic devices, and use of artificial organs. (4) Monitoring systems--the application of engineering theory to research on problems associated with the diagnosis and management of the critically ill patient is of prime importance. In this, projects will continue to be supported that are devoted to transducer development, hybrid processing units, and reliable systems for data acquisition, diagnosis and display.

During the past few years the Institute has considered the desirability of the development of Centers in Biomedical Engineering. Over a period of time such centers would allow us to (1) define more clearly the interrelationships existing between engineering and bio-medicine; (2) delineate more sharply the characteristics and limitations of modern engineering and the means by which our national engineering capability can more effectively be directed toward fundamental and applied biomedical problems; (3) define program opportunities and needs more specifically on the basis of emerging engineering theory and practice

and the applicability of these to problems of biology and medicine; (4) make a continual assessment of the areas of greatest potential for the application of engineering concepts to biology and medicine; (5) design more effective administrative frameworks for biomedical engineering activity; and (6) provide a setting for a meaningful and productive evolution of national policy and action in this area. There is no question but that the conversion of medicine from an art to a science requires the development of whole new arrays of quantitative techniques and modes of analysis and treatment. Toward this end the establishment of biomedical engineering centers can optimize the opportunity for the successful application of the concepts, methodologies, and techniques of engineering science to biomedicine. The Institute, with encouragement from the Congress and from its Council, intends to initiate steps for the establishment of one or two biomedical engineering centers during the coming year.

From time to time, the question arises as to examples of biotechnological innovation, supported by the Institute, that have effectively been put to use in the delivery of medical care. This year we have chosen to cite several examples of such successful innovation.

1. Hospital Computer Project--Massachusetts General Hospital
   GM 15287 (Barnett)

   This project was initiated in 1963 to develop computer techniques to improve patient care and to provide new methods for performing research on the information in the medical record. One of the major accomplishments has been the development and implementation of a time-sharing computer system and programming language called MUMPS (MGH Utility Multi-Programming System) which facilitates access to various types of hospital records. Through its use, the hospital data base is accessible at all times to all users. The system is now being marketed by several commercial firms for use by other hospitals.

2. Biomedical Engineering Program Project--University of Southern California
   GM 16437 (Grodins)

   Measurement of cardiac output is extremely important in the care of seriously ill patients, particularly those with respiratory and cardiac disorders. Ordinarily an invasive technique is required for the measurement of this parameter. Dr. Grodins has developed a mathematical model of respiratory control, which includes cardiac output as one of its components, and which permits the measurement of this important physiological activity without recourse to invasive techniques. This technique is now routinely proving extremely useful.

3. Bioengineering Research in Neurosciences--University of California, Los Angeles GM 16058 (Adey)

   Symptoms of many disorders, such as epilepsy, do not necessarily occur in the hospital but may occur at times and places when measurement of the parameter characteristic of the disorder cannot be made. A system for transmitting of electrical signals characteristic of certain disorders from remote locations by telemetry and telephone, has been developed at the Brain Research Institute of the University of California, Los Angeles. The system has already been applied to the study and treatment of epilepsy.

4. Mathematics in Biology and Medicine--University of Southern California GM 16197 (Bellman)

The determination of the optimal dose of a drug to use in the treatment of a specific patient is difficult at best, and has ordinarily been established on a trial and error basis over a long period of time. A relatively simple, rapid, computerized system for determining the dosage of digitalis for heart patients has been developed at the University of Southern California by application of a mathematical model for the kinetics of digitalis absorption and excretion.

Training

These grants provide support for research training in biomedical engineering ranging from studies involving quantitative control systems analysis, prosthetics, information systems and fluid mechanics, to bio-instrumentation. Initially the program area was established in 1962 with awards totalling $144,407 to the University of Rochester, Johns Hopkins University and the University of Pennsylvania. With careful selection of new awards, the program was expanded to 19 grants by FY 70. The predominant base of biological support is in departments of physiology. The program content now ranges widely: MIT is primarily involved in communications engineering, Northwestern University is a university-wide center incorporating bio-instrumentation and physiological control systems, and Johns Hopkins is heavily oriented toward the study of neurophysiological functions.

Currently there are 18 programs and they are housed in engineering schools (12), medical schools (4), and jointly between a medical and an engineering school (2). The total predoctoral enrollment was 235. From this group some 40 Ph.D.'s have been awarded.

Two interconnected difficulties face biomedical engineering as an emergent discipline: 1) the problems of soundly integrating the biological component as well as advanced engineering into balanced graduate curricula, and 2) the question of the significance of some of the specific research to solutions of problems of pressing biomedical importance. To meet these problems, changes in curricula are being developed slowly but effectively. The Training Committee has been active in forecasting trends in biomedical engineering research and development, which will produce individuals who have the originality and fundamental nature requisite for Ph.D. and postdoctoral training. Also, there is steadily increasing interest in schools of engineering and medical schools for developing sound programs in biomedical engineering. These elements form the basis for future development in this emergent discipline.

In Fiscal 72, the Engineering in Biology and Medicine Training Committee made an effort to project the national manpower needs in biomedical engineering during the decade to come and to assess the impact of different types of federal support of graduate training in biomedical engineering. The Committee believes that, although there may be an apparent oversupply of biomedical engineering Ph.D.'s during the next year or two, in the longer range the need for biomedical engineers at all educational levels will meet or exceed the capacity of the nation's educational system to produce them. This view

depends, however, on the assumption that certain changes will take place, both in the biomedical engineering industry and in the universities. The Committee concluded that a balanced collection of several means for governmental support of biomedical engineering graduate students is both appropriate and necessary.

Research Highlights

1. Ko - Case Western Reserve University - GM 14267-06
"Microelectronics Laboratory for Biomedical Science"

Continuous surveillance of intracranial cerebrospinal fluid pressure is critical in the hydrocephalic patient. In treating this disease, accumulation of fluid in the cranium is prevented by surgical placement of artificial fluid shunts. These shunts, however, are prone to malfunctions, resulting in the accumulation of unwanted fluid and increased pressure. A method of checking malfunctioning shunts is therefore as important as the shunt itself.

A portable instrument has been designed in the Microelectronics Laboratory for Biomedical Sciences which is capable of monitoring pressure in small catheter probes. These probes can be placed in areas of the brain where fluid and pressure build-up occur. A simple periodic check of pressure in the hydrocephalic patient readily discloses the effectiveness of shunting procedures.

A new type of pressure monitoring system is currently in development which will be capable of providing continuous and fully automated operation. It will employ an ultraminiature electronic pressure sensing device which, when implanted along with a shunt, will continuously monitor intracranial pressure. The device can be used to regulate flow in auxiliary shunts or to warn the patient or medical attendant of the onset of improper drainage. This pressure sensor is also ideally suited to other clinical and research applications.

2. McKeown - University of Chicago - GM 17292-02
"Computer Monitoring in Obstetric Intensive Care"

Most fetal-maternal monitoring systems currently in use display data but do not issue warnings of possible intrapartum pathology; thus, effectiveness is dependent upon training of skilled observer personnel. A truly effective monitoring system should issue warnings of existent or impending abnormal conditions in a manner easily understood by health care personnel at all levels of training

In the obstetrical monitoring system at the University of Chicago, a small digital computer has been introduced which analyzes and correlates data for graphic display of routine and warning information. Signals representing fetal cardiac activity and maternal uterine dynamics are analyzed. Raw and analyzed data are stored in the computer system. Abnormal contraction pressure, nonspecific fetal bradycardia, early, late and variable fetal heart rate deceleration trigger warning messages and audible alarms.

The observer can interact with the system through a small function keyboard. This allows initial calibration of the system and optional displays. These include a digital readout of fetal heart rate and uterine pressure at any instant. A trending analysis of up to 28 hours of monitoring data can be displayed.

3. Peters - University of California, San Diego - GM 17284-03
"Computer Monitoring of Surgical Patients"

Acute respiratory failure is one of the major killers in our society. In accidental trauma, particularly highway accidents, only head injury is more lethal. In patients who have had major surgery, acute respiratory failure is the primary cause of death. At present, methods of monitoring the state of the lungs are very limited. Routinely, only respiratory rate is wanted and a subjective evaluation of respiratory distress. In the last 10 years, the improvement in methods of measuring arterial blood gases has permitted identification of patients who have developed respiratory failure. This has greatly improved treatment but is a late change and does not identify the nature of the malfunction.

At the University of California, San Diego, investigations of means of monitoring respiratory function have focused on a solution to this problem. A system has been developed for measuring the work of breathing, the stiffness of the lungs, and the efficiency with which oxygen and CO_2 are exchanged through the lungs. This is designed for use in an intensive care unit on critically ill patients.

All measurements of such lung function require computations to provide useful clinical information. Therefore, such a system must provide a means of collecting data from critically injured patients, transmitting the output of measuring instruments to the user. The system to accomplish these tasks has been designed to be cost effective by remote communication for community hospitals.

Criteria have been developed to determine whether a respirator is indicated to breathe for the patient or when a patient on a respirator can safely be removed. Early recognition of respiratory deterioration permits early treatment. This shortens the period of respirator use required and diminishes the risk of death.

Contracts

1. "The Development of a High Voltage Electron Microscope"
Contractor--Atomic Energy Commission (Oak Ridge National Laboratory)
Funds Allocated--$100,000

Dr. Norman Anderson, of the Oak Ridge National Laboratory, is currently directing our collaborative program with the AEC for the development of a high voltage electron microscope capable of observing macromolecule structure. To date these efforts have succeeded in perfecting a functional 150 kV microscope with an excellent vacuum chamber and a cryogenic sample stage. The investigators are now assemblying an instrument that should be

capable of achieving 5 Å resolution using samples stored at liquid helium temperatures. The microscope as presently assembled has two potentially attractive features: 1) an extremely coherent source which allows the extraction, by computer, of data far beyond its actual resolving capability; and, 2) specimen lifetime can be prolonged because of the absence of sample contamination and the achievement of low specimen temperatures.

The Institute, over the past five years, has developed a $1 million program in the electron microscopy area. This contract is complementary to the overall program. Projected tasks for the remainder of the contract period include testing of the support for the microscope and further assessment of its biological applicability. It is anticipated that the Institute will continue support of this instrumentation development through the initial evaluation of its effectiveness with respect to macromolecules.

B. Automation of Clinical Laboratories

Research

By 1975 the nation's clinical laboratories will be faced with the performance of some billion tests per year, and a likely insistence that quality be markedly increased and that the cost per test be decreased. It is clear that the only solution to this critical problem is to find ways to mechanize and automate the clinical laboratory. The solution is not an easy one as it involves an extremely complicated mixture of activities in clinical chemistry, hematology, microbiology, mycology, parasitology, serology, and urinalysis. It has become apparent that a variety of bottlenecks and unsatisfactory techniques in instrumentation and data processing are seriously retarding the realization of completely automated systems. To help resolve this situation, the Institute has undertaken a concerted program of research and development in this area.

Within this program a remarkable new piece of equipment, conceptualized by Norman Anderson of the Oak Ridge National Laboratory of the Atomic Energy Commission, has been carried through to reality and use in the clinical laboratory in the past year by an integrated effort on the part of representatives of government, federal laboratory, university and industry.

Basically, this "Fast Analyzer" is a new microanalytical system which employs a rotor containing 15 reaction chambers or cuvettes, spinning past a beam of light. Standards, samples, and reagents are placed in a central loading disc--and all solutions move out into the reaction chamber by centrifugal force. In practice, many clinical chemistry methods may be completed in as little as 30 seconds after the rotor has started. The data are recorded photographically. By means of a computer, calculations are carried out instantaneously and a printout of valid data is provided by the physician within minutes of his request instead of hours. The system's principal impact has been to decrease the unit cost per test and to increase by manyfold the rate at which analytical chemistry analyses may be performed.

Following a concentrated effort in research and prototype development and testing, industry was brought into the picture at an early date. Enthusiasm among industrial organizations was high. Royalty-free licenses were granted

by the government to individual commercial concerns with the result that in a remarkably short period of time the instrument has been made available to our clinical laboratories. The major triumph here has been in the management of the innovative process that lay between the idea and its application.

At a recent scientific meeting of the American Association of Clinical Chemists, the "Fast Analyzer" was cited as the outstanding innovation in automation of the clinical laboratory over the past 15 years.

In the summer of 1971, a White Paper Report for clinical laboratory sciences was developed. Future goals, priority of needs, and strategy, were set down as guidelines to the Institute staff and committee members in considering contract and grant proposals.

In the fall of 1971, a status report of the Automation in the Medical Laboratory Sciences Review Committee was completed. The booklet, titled "The Mechanization, Automation, and Increased Effectiveness of the Clinical Laboratory" is an overview of the field of clinical laboratory sciences and it cites those areas in need of more research and development. The report assesses the status of the clinical laboratory sciences in terms of emerging trends and opportunities. It points to specific needs and identifies neglected areas. Major areas of interest are clinical chemistry, hematology, microbiology, virology, and immunology.

Four steps were recommended for a systematic approach to automation and the delivery of uniformly high quality, low cost laboratory services:

1. Upgrade existing systems and methodologies as an interim solution to current overwhelming increases in hospital laboratory workloads.

2. Apply existing knowledge more thoroughly in the development of new methods, procedures, instrumentaton and systems.

3. Support basic research and development in areas of high promise.

4. Develop standards for reference materials, equipment calibration and other measures of uniformity to assure validity and reproducibility of laboratory testing.

Training

The aim of the clinical chemistry training program is to prepare trainees for careers of teaching, investigation, and service in the field of clinical chemistry. Particular attention is given to meeting the acute shortage of trained personnel in the clinical laboratory. The training program is closely aligned with the Institute's automated clinical laboratory research and development program. Clinical chemistry training programs have been based in departments of biochemistry and pathology but must often combine the activities and facilities of both departments.

The training program began in 1964 with the award of one postdoctoral program at the University of Washington. A second (predoctoral) was started in 1966. All of the current programs (7) are at medical schools or, in one case, a large teaching hospital.

The trends for the future in clinical chemistry are already set. An increase in the quantity and quality of health services required has begun to develop. Partially automated laboratories exist and second generation computer operated facilities are being constructed. Regional facilities are being considered to serve large metropolitan areas. The operation of clinical laboratories is becoming much more complex and the need for trained personnel is acute. The Institute is concerned not only with the development of the automated clinical laboratory but also with the training of personnel to staff and develop these facilities. We will continue to encourage applications for training in this area.

Research Highlights

1. Herzenberg - Stanford University - GM 17367-03
"Automated Cell Sorting-Clinical and Biological Uses"

Dr. Leonard Herzenberg and his associates are developing drop deflection techniques and instrumentation, including immunofluorescent detectors, for automated sorting of functionally different living human and other mammalian cells. In its simplest terms, the technique involves suspending human cells in a life supporting fluid which is then treated with dilute solutions of fluorescent dyes. The solution containing a variety of cells is then directed down through a narrow tube where a detector senses those cells which fluoresce. On signal from the detector, a magnetic bias is imposed on the stream of solution momentarily causing it to deflect--depositing the drop of fluid containing the "tagged" cell in a special receptacle for collection. These studies have made possible many direct applications to clinical and laboratory work. The immunofluorescence cell studies have already provided an insight into many poorly understood details of the immunological system. For example, it now appears that the precursors to antibody producing cells carry immunoglobin and specific antigen binding markers which require activation to enable them to differentiate into actual antibody producing cells. Development of successful semi-automated methods for such clinical applications as tumor detection, reticulocyte counting, malaria detection and differential leukocytes counting give promise of extreme usefulness in areas where automation has, heretofore,had very little impact.

2. Leif - Papanicolaou Cancer Research Institute - GM 16476-02
"Electronic Cell Volume Analysis"

The first stage of a complex new device called AMAC (Automated Multi-Parameter Analyzer for Cells) has been completed. Basically, this system allows Papanicolaou Test Cells suspended in a supporting fluid to pass single file through a sophisticated new kind of sensor that will measure a variety of characteristics of each cell. Each cell is in the sensor for only 10 millionths of a second. But a laser, flashing 4,000 times a second, will flood each cell with light.

Cancer cells and pre-cancerous cells, theoretically, have a slightly different way of scattering the light than normal cells. As soon as a particular type of cell's "profile" is determined, an analyzer, attached to a computer, is able to pick out suspicious cells and alert technicians to give that particular sample a close visual examination.

The device should be able to examine at least 250,000 cells per minute. The sensor will also examine the total volume of key substances like DNA and RNA for possible clues to the cell's state of normality or malignancy. The results will be in terms of numbers and measurements which can be fed into a computer. The computer, programmed to watch for certain patterns, should be able to screen out 90-95 percent of the normal cells virtually instantaneously.

3. Savory - University of Florida - GM 17203-03
"Clinical Analyses of Trace Metals by Gas Chromatography"

A novel gas chromatographic procedure for the measurement of chromium in serum has been developed. In this method, chromium is co-precipitated with serum proteins and extracted from the precipitate as chromium trifluoroacetylacetonate which is measured gas chromatographically using electron capture detection. Recoveries average 75% and normal values range from 0.4 to 1.8 µl per 100 ml.

Measurements of levels of chromium in biological materials are important from both a toxicological aspect, and from the functioning of chromium as a trace element essential to normal metabolic processes in man. Dr. Savory and his group of investigators have found high serum chromium levels in diabetics and renal transplant patients.

Gas chromatography is a specific and extremely sensitive method for the detection of metals as the Beta-diketone chelates. It is just coming into popularity in the clinical chemistry laboratory and represents one of the truly exciting advances in the field of separations chemistry.

Contracts

1. "Fast Clinical Analyzer"
Contractor--Atomic Energy Commission (Oak Ridge National Laboratory)
Funds Allocated--$198,000

This interagency agreement has produced a computerized, centrifugal instrument that is being used for blood chemical analyses in the clinical laboratory. The instrument (GeMSAEC) can produce precise analysis of up to 14 blood samples run simultaneously. Previously, enzyme tests for the differential diagnosis of heart, liver, and kidney problems of patients took hours. The same tests now can be performed in minutes on this computerized analyzer. The development of a miniaturized model promises a substantial reduction in cost to small hospitals and laboratories and useful portability.

The miniaturized version will be carried to a sufficient state of development to permit industry to produce reliable working machines. Chemical

procedures need to be adapted for micro-quantities of sample and reagent, procedures developed for production of disposable low-cost molded rotors, and the instrument itself must be field-evaluated in several clinical laboratories.

2. "High Resolution Analytical Techniques"
Contractor--Atomic Energy Commission (Oak Ridge National Laboratory)
Funds Allocated--$300,000

The Body Fluids Analysis Program at Oak Ridge National Laboratory has developed a high pressure liquid chromatography system to separate and identify compounds from biological fluids on the basis of their UV absorbance. Major developmental efforts to date have been focused on the analysis of low molecular weight constituents in urine. This instrument provides the means for separating the more than 500 molecular components obtained from biological fluids (blood or urine) into individual peaks which can then be identified by other means. This past year, new metabolic pathways for orotic acid were discovered at Duke University School of Medicine by use of the UV analyzer. There are three prototype instruments in the field at present: Clinical Center, NIH; Duke; and Johns Hopkins University School of Medicine.

This work on high resolution automated analysis is complementary to the overall programs of the Institute. Since most diseases are believed to be caused at the molecular level, such analyses will be important to modern biomedical scientists in defining the causes. Continuing activities will include further identification of unknown separated constituents by spectral and chemical means and the development of data processing programs. Three data processing systems, each including a mini-computer and interface, will be installed at selected clinical laboratory sites for rapid interpretation of analyzer tracings.

3. "Standard Reference Materials"
Contractor--National Bureau of Standards
Funds Allocated--$657,000

The aim here is to improve the accuracy of measurement in clinical chemistry tests by providing standard reference materials for clinical analytical procedures. This interagency agreement has to date produced 16 different standard reference materials which are contributing to the accuracy of laboratory results. Two national standards have greatly improved the accuracy and reliability of determinations of triglycerides and cholesterol in blood. The determinations are used to make diagnostic and therapeutic decisions in cases concerning heart and blood vessel diseases.

Further standards are being developed that will be of importance both to specific chemical tests and also to the standardization of clinical laboratory instrumentation.

4. "Sample Identification"
Contractor--Georgetown University
Funds Allocated--$143,055

A major problem in the clinical laboratory is the proper identification of specimens with the patient (or source) from whom they were obtained. Georgetown University and American Science and Engineering Co. have developed and established proof of principle for a unique working system.

The contractor intends to evaluate the prototype instruments developed for this process by the American Science and Engineering Company. They will integrate this method with a patient sample identification system in the laboratory, and develop and evaluate an encoding mechanism which will automatically identify patient, patient's sample, and tests to be performed. The present intent of the Institute is to pursue the objectives of this contract through 1975.

5. "Sample Identification"
Contractor--American Science and Engineering Company
Funds Allocated--$372,686

The contractor proposes a three phase program. Phase I culminates in the delivery of a prototype sample identification system suitable for field testing at the Georgetown University Hospital. Phase II continues the field test through whatever design modifications are required and culminates in the delivery of the automated admission unit and the full complement of inpatient bedside modules. Phase III covers the work in which the outpatient and emergency units are delivered and a full scale test of the entire system is performed.

The present intent of the Institute is to pursue these objectives through 1974.

C. Biometry

Training

The peak year of support for biometry research training was 1965 when there were 39 active grants in Schools of Public Health (13), Schools of Medicine (8), and Graduate Schools (18). Currently (1971-72), the 20 active grants are distributed between Schools of Public Health (5), Schools of Medicine (3), and Graduate Schools (12).

The goals of the program in Biometry have been training for careers in teaching and research in an academic environment or pursuit of cooperative research in national health-oriented research agencies such as NIH. Many current programs include modern computer techniques, and increasing emphasis on mathematical modeling and biomathematics applicable to medical problems as well as general biostatistics.

Because of the extreme manpower needs for biostatisticians, the trainees in many programs terminated their formal training with the Master's degree,

especially in the earlier years. The reviews of renewal applications, however, have exerted rigor in recommending support for only those grants which have developed sophistication in curriculum and adhered to the original goals of training doctoral candidates. As a result, there has been a diminution in the number of active grants to 20 during the 1971-72 year.

The need still exists for biometricians who possess sound backgrounds in appropriate statistical techniques as well as biological orientation sufficient to permit them to truly collaborate in the solution of medically important problems. Indicative of the unsaturated state of the field is the fact that trainees finishing the program in June or August, 1971 each received several job offers from which to choose. Further evidence of manpower shortage is indicated by the numerous basic science departments which find it necessary to provide courses in Biometry relevant to their disciplines and taught by their own faculty because of inability to obtain or support full-time biometricians. This stop-gap procedure leads to handicapping the generations of scientists now in training by providing less than optimal preparation in Biometry. The number of high-quality sites where biomathematicians seeking careers in medical research can be trained are very few at present. Therefore, future efforts of this program will be directed to the identification and stimulation of additional training sites for the training of greater numbers of biometricians with career orientation toward the medical sciences.

II. Program Administration

Automated Clinical Laboratories

A major administrative difficulty lies with the review of research grant applications in the automated medical laboratory sciences area. For a time, within the Division of Research Grants, there was an Ad Hoc Review Committee for these applications. However, this review group has been discontinued. Accordingly, applications pertinent to the Institute's programmatic interests and responsibilities are often reviewed without any specific sense of continuity and often without any real relationship to each other in a program sense. We believe that it might be appropriate to consider that these research grant applications undergo scientific review by The Automated Medical Laboratory Sciences Committee of the Institute. Such a policy might be implemented by making the services of this Committee available to the Division of Research Grants on an ad hoc basis.

Biomedical Engineering Centers

Over the past two years there has been intermittent discussion within the Institute on the matter of the possible establishment of biomedical engineering centers. Many difficulties presented themselves, such as questions as to what constitutes "a center," and the matter of definition with respect to a "biomedical engineering center," especially from the point of view of its relationships to industry and to services it might conceivably provide to a surrounding medical care community. Dr. Stetten, Director of the Institute, initiated a thorough discussion on the matter of Centers in general in 1972 (See Director's Report), and this paved the way for a more detailed evaluation of the intent and content of possible biomedical engineering centers. The Institute's Council also elected to engage in an evaluation of the centers'

concept and the financial problems they present. It was possible during a round of the Council's examination of this topic to lay out the history of the development of the interest in biomedical engineering centers and to specifically discuss the matter of policy development and priority setting with respect to them. Following this, the Senate Appropriations Committee, noting that the Institute's Engineering in Biology and Medicine Program has matured considerably over the past 10 years, suggested that it is timely to consider the initiation of a Biomedical Engineering Centers Program over the next several years. It is likely that this program will now be undertaken gradually.

Clinical Engineers

The character of the Institute's Engineering in Biology and Medicine Training Program reflects the main mission of the Institute in that it is directed towards preparing students for careers in research and teaching--towards the production of Ph.D.'s who will be able to serve as independent scientists in academia. The program has not been directed to the production of medical or clinical engineers who would engage primarily in the delivery of medical care. However, with an increasing interest in engineers participating on the medical care scene, there has been rising interest in the training of clinical engineers and in the past year, this matter has been under review. It is likely that, if a substantive commitment were to be made to training in this area, responsibility for the administration of it would fall to either the Bureau of Health Manpower Education or the Health Services and Mental Health Administration.

GENETICS PROGRAM

I. Program Content

A. Introduction

The NIGMS program in Genetics is directed toward gaining a better understanding of the processes and mechanisms of inheritance. Its objectives are the prevention, therapy and cure of genetic diseases in man. This includes not only those diseases completely under genetic control but also multifactorial diseases with a strong hereditary component, such as diabetes, atherosclerosis, hypertension, and schizophrenia. The problem of human genetic disease is treated as a continuum, suitable to a generalized approach. Discrete human heritable diseases are studied as model systems, yielding concepts capable of wider applicability.

By contrast, the categorical institutes of the NIH appropriately tend to consider the treatment of each individual genetic disease of specific program interest to them as a discrete medical problem, demanding its own approach and resolution. The program interest of the NIGMS in genetics and the interests of the other institutes in specific genetic diseases are thus seen as complementary. The distinction has been made that there are appropriate interests of the several categorical institutes in one or another genetic disease; in a broader sense, then, this Institute is concerned with the genetics of disease, and also with generalized approaches to detection and therapy.

The concept of this program implies broad concern with man's overall genetic constitution and the rules that determine its varied expression in health and disease, as well as consideration for man's genetic future. Much remains to be known. We continue to need simpler experimental models than man in order to progress. Accordingly, a substantial portion of the NIGMS supported research in Genetics continues to exploit such systems as bacteria and their viruses, yeasts, fruit flies, the mouse, and human and animal cells in tissue culture. In addition, the Institute supports studies on both human and animal population genetics. This research is regarded as a resource for the generation of concepts ultimately applicable to human genetic disease.

In the past few years, we have noted a striking increase of interest and of excellent applications in the genetic problems of man. This trend can be attributed to several developments including: (1) Improvement of technology, particularly in tissue culture and somatic cell genetics, (2) A desire and increased capability by investigators to extend and perhaps modify the conceptual framework based on lower model organisms to human genetics, (3) The realization by "basic scientists" that such studies are of direct relevance to current medical problems, and (4) Increased interdisciplinary interactions, particularly of biologists, biochemists, and geneticists, with research clinicians.

B. Trends and Needs in Genetics

1. Interdisciplinary Research: The increased emphasis on human genetics requires greater input from a variety of disciplines, including medicine. Genetics deals with inherited variation. In man, most of the variations are diseases producing unpleasant symptoms or unwelcome structural abnormalities. The agent producing these effects is a mutant gene or sometimes, more grossly, a chromosomal anomaly. Some of the heritable disorders are common; many are rare. The latter are important out of proportion to their numerical incidence, since, like mutations in lower organisms, they can often shed light on normal function. For example, the relation of glycolipid metabolism to nerve function, or some details of the mechanism of blood clotting, first became known through studies of abnormal conditions, such as Tay-Sachs disease, or hemophilia, in which a specific biochemical reaction is faulty owing to an inborn metabolic error.

The experimental material of human genetics is often therefore the victim of genetic disease. He is, as a patient, the object of medical care. At the same time he is a scientific subject for investigations seeking to alleviate his malady. The medical ethic demands that the two aspects not conflict. Thus, team effort between the clinician and a large variety of more basically oriented disciplines is required. Both groups stand to benefit from intense exposure to each other, and from collaborative effort. The clinician benefits by acquiring fundamental tools applicable to diagnosis and treatment. The biologist, biochemist, etc., who has heretofore often been exposed only to simpler organisms, benefits by recognizing the rich variety of human mutations available for fundamental genetic analysis. This recognized need for greater interdisciplinary interactions in human genetics forms an important conceptual aspect of a new NIGMS effort to establish Centers in Human Genetics.

2. Tissue Culture: The intact human being is far from an ideal subject for genetic studies. Traditional methods, such as controlled genetic crosses, are not possible. In addition, a generation time of 20-30 years is hardly convenient, even for the most patient researcher. Thus, the student of human genetics has, up to recently, had to rely on family pedigrees in order to obtain clues about the detailed mechanisms of human inheritance. It is therefore not surprising that we know far less about our own genes than those of the common bacterium E. coli or those of the laboratory mouse. Advances in tissue culture are now promising to provide a powerful adjunct to the study of human pedigrees. The new field of somatic cell genetics offers an approach to the "mapping" of human genes via tissue culture techniques.

The methods of somatic cell genetics rely on the phenomenon of interspecific cell hybridization. For example, mouse cells and human cells, each separately grown in tissue culture, can be made to fuse, forming mouse-human cell lines, in which each hybrid cell has a single nucleus containing both mouse and human chromosomes. Upon continued growth of such hybrids, human chromosomes tend to be eliminated. Concomitant

with the loss of some human chromosomes, a parallel loss of human functions (i.e., enzymes that can be identified as of human origin) occurs. The retention or loss of identifiable human chromosomes (another very recent notable development) can be correlated with retention or loss of specific human functions. In this way, we can now begin to assign such specific functions (genes) to specific chromosomes.

It is difficult to overemphasize the importance of this new development in relation to human genetics and human genetic disease. The history of genetics has analogs with other forms of exploration: The chromosomes of the cell's nucleus, like any terrain, must be mapped before they can be further understood.

Advances in the art of tissue culture have had other important effects on the study of inherited human diseases. We can now study many such diseases in cells derived from patients. In the past year, the specific molecular defect of several additional genetic diseases has been identified through tissue culture methods. Furthermore, new strategies for therapy can often be explored and developed through the use of tissue culture, thus preventing needless trauma to patients.

The detection of genetic disease in the unborn, as well as the detection of potential for such disease in "carriers" is a relatively new development (see below) that has also arisen from progress in the culture of human cells.

3. Genetic Counselling: Any serious medical disorder experienced by a family may raise realistic or unrealistic fears that the disorder is genetically determined, particularly if the disorder has affected more than one family member.

These fears of genetic abnormalities often remain undetected and unresolved. If family members wish to obtain genetic information, they may seek information and guidance from medical or non-medical sources. This information, if erroneous, is potentially harmful. When a family asks for genetic advice from their physician, and their questions and anxieties remain unresolved, the need arises for expert genetic counselling services.

The growing recognition that the unique services of the genetic counsellor will be required by increasingly large numbers of people is reflected in a variety of ways. Among these is concern that a sufficient number of practitioners of this new medical specialty will be trained during the present decade. In his congressional testimony in the spring of 1972, Dr. DeWitt Stetten, the Director of the NIGMS, stated:

> "There are less than 100 qualified medical geneticists with combined medical and genetics training in the United States at present -- this is about one per medical school. A reasonable estimate is that about three more per school, or about 300 more medical geneticists, are needed for existing medical schools; about 60 are needed for new medical schools

now forming; and about 50, or one per school, are needed for dental schools. This means that about 400 more medical geneticists are needed for medical and dental schools. These figures do not take into account the need for about 250 medical geneticists for counselling centers in hospitals which are not otherwise affiliated with medical schools, and about 100, or about two per state, for each state board of health. The total need is thus about 750. These needs are urgent but cannot be met at once, for the number of qualified training sites is few and the number of qualified candidates interested in a career in medical genetics is limited; these numbers are increasing, however, as more physicians and scientists are becoming concerned with the problems of medical genetics and are seeking additional training."

The need for the qualified genetic counsellor will continue to increase in proportion to the power of the techniques available to him. It is well known that the carrier (heterozygote) state for a number of genetic diseases can now be accurately diagnosed. Indeed, screening programs to detect carriers of sickle cell disease, and Tay-Sachs disease are now underway in a number of communities. The number of disease states that can be detected, and the numbers of families known to be at risk for specific genetic diseases will continue to increase.

Coupled with more effective heterozygote detection methods are continuing and marked improvements in the detection of genetic diseases in the unborn, through the technique of amniocentesis. This is a method in which free-floating cells, withdrawn from the fluid surrounding the fetus are cultured and then tested for genetic abnormality or disease. When a defect associated with severe disease, early death or severe mental retardation is diagnosed, the parents can be given the option of terminating the pregnancy. In other diseases (e.g., galactosemia) prenatal diagnosis offers the possibility of early diagnosis, and treatment prior to birth. There are a fairly large number of chromosomal abnormalities (e.g., Down's syndrome, or "mongolism") that can be detected. In addition, some 45 familial metabolic disorders can now be demonstrated in tissue culture, and the enzymatic characteristics of nearly half of these have been detected, to date, in amniotic fluid cultures. Couples with a high genetic risk are thus enabled to have normal children; without this safeguard, many would not have risked conception.

4. Opportunities in Treatment and Prevention. Enzyme Replacement and Genetic (DNA) Therapy: Some genetic diseases lend themselves unusually well to ingenious employment of therapeutic maneuvers, and often the results are gratifying. Metabolic defects can often be overcome by supplying a missing metabolite, (thyroxine, in metabolic cretinism), by a vitamin (pyridoxine, in homocystinuria), or by limiting the intake of a precursor which may undergo toxic accumulation (diets low in phenylalanine, in phenylketonuria). The accumulation of toxic metabolites can be prevented through the use of metabolic inhibitors

(allopurinol, to reduce uric acid formation in gout) or by strategies of depletion (removal of excess copper by penicillamine, in Wilson's disease). These treatments do not correct or restore the missing function, but rather serve to ameliorate its effect. In principle, one could also restore function by supplying a protein or enzyme that is missing or defective. Another more distant possibility is to supply the missing or defective gene, by administering functional DNA.

A limited number of inborn errors can already be treated by administration of a missing enzyme or protein. Examples include treatment of agammaglobulinemia with gamma-globulin, and of hemophilia with antihemophilic factor. A classical example, still surprisingly poorly understood, is the treatment of diabetes with insulin. A potentially important variant is the use of organ transplantation in the treatment of a number of metabolic diseases. The number of metabolic diseases whose cause is known to be a defect in a specific and well-understood protein is rapidly increasing. While, the field of enzyme replacement therapy is still in an embryonic state, there have been partial successes in model systems, such as tissue culture.

In some cases of enzyme deficiency, abnormally low amounts of a functional protein are made. In others, the rate of enzyme degradation, rather than the rate of enzyme synthesis may be affected. Therefore, some attention is being given to stabilizing endogenous enzymes against degradation, or to supply extraneous enzymes in a more stabilized form.

Most of the successful examples of replacement therapy have been applied to proteins or enzymes found in blood. The actual defects in most metabolic diseases, however, are intracellular. Therefore modification of the proteins for use in therapy is also being considered in order to introduce them into the appropriate cell, or even into cell organelles. For example, specific glycosylation of an enzyme or protein, so that it is recognized only by the appropriate cell, may allow for more specific uptake.

A more distant and difficult goal is the insertion of specific genes into human cells and perhaps, ultimately into the cells of patients. While the problem has intrigued molecular biologists for some time, prospects for application to human therapy appear, at this time, remote. The NIGMS is now beginning to receive and fund an increasing number of applications dealing with this research topic. The prototype for the mammalian cell is the bacterium E. coli, in which specific genes can readily be added in a variety of ways from the outside of the cell, resulting in permanent genetic alterations. By analogy, there are also a number of possibilities for introducing genetic material into human cells: Introduction of DNA or of chromosomes either directly or by somatic cell fusion; transfer of genetic material from one host to the other by virus-like particles containing DNA of the host cell; infection with active or inactive viruses containing genes that can determine some biochemical function; or infection with a viral nucleic acid to which some cellular gene has been coupled. There have been some partial successes of such techniques in tissue culture: a chicken

chromosome has been added, by somatic cell fusion, to mouse cells, correcting a genetic defect in the latter; the enzyme thymidine kinase has been produced in deficient mouse cells after infection with an inactivated virus; and a bacterial virus has been shown to be capable of introducing bacterial genes into human cells.

Gene therapy would, in principle, involve isolation of somatic cells from the tissues of a diseased individual, alteration of their genetic endowment in the test tube, and replacement into the individual. In practice, many feel that such techniques would have severe limitations, and that only a small fraction of inborn errors could be helped. With new developments in the understanding of the immune response, such disorders may be more readily treated, in the future, by tissue and organ transplantation, or some type of enzyme replacement therapy. The real potential of this work is that it could lead to new and powerful research tools, which in turn may contribute to greater understanding of human heredity.

5. Social and Ethical Aspects: There is now a growing public and professional recognition of the ethical, social and legal problems arising from recent developments in genetics. Modern developments, including a new potential for genetic manipulation, challenge accepted attitudes about the inviolate nature of man's inherited endowments. The subject has raised both fears and hopes - fears of a new technology without the social and political resources to utilize it wisely, hopes for eradication of serious diseases. The topic has been raised in Congress. Senator Mondale has introduced a resolution to establish a commission to investigate the ethical, legal, and social aspects of medical research and practice. The NIH has also expressed its concern, through regulations to protect the rights of subjects in human experimentation, through a review process which highlights areas of social and ethical impact in research proposals, and through sponsoring symposia devoted to such issues in genetics research.

In the public mind, these concerns are often expressed as fears of the creation of human genetic carbon copies through "cloning" or in vitro fertilization of human ova, with subsequent reimplantation into the original or a surrogate mother. The term "genetic engineering" brings to mind both therapeutic and eugenic aspects of manipulative genetics, using the tools of molecular biology. It is far more often used in the popular literature than "genetic therapy", a more limited aspect referring to the correction of genetic disease at the level of the individual patient. The time scale within which scientific advances might be translated, if unchecked, into technology is thought of in terms of decades, or even centuries.

Although the Institute is also concerned with possible long-range misuse of genetic knowledge, it is felt that other and more immediate issues raised by modern genetic medicine should receive the bulk of its attention. These issues include, for example, the impact of genetic counselling and mass screening on the individual, the family, and society. Advances in genetic knowledge, increased public demand, and refinements in amniocentesis have all helped to account for a

sudden spurt in genetic counselling. This, in turn, has served to emphasize the recurring dilemnas facing the genetic counsellor today. Among these are conflicts in the rights of the individual to privacy, and the rights of society. For example, the law does not require an individual to make known the fact that he harbors "deleterious" genes, nor does it require the physician to inform other family members. Should such revelation be required, in view of the vertical transmission of disease traits, from parents and ancestors, through the individual and his siblings, to their descendents?

Another current problem facing the genetic counsellor turns on different theories and styles of counselling. His traditional role has been that of the "neutral educator", doing no more than presenting the statistical probabilities and facts about various diseases and their severity. In fact, it appears that counsellors influence decisions by the way in which they present information and describe available options. Often covertly, and sometimes overtly, they have a strong influence on family planning. What if some of the options presented conflict with the religious and moral values of patients, as in the case of therapeutic abortion?

Mass screening programs for sickle cell trait, sickle cell disease, and the carrier state for Tay-Sachs disease, have been initiated in recent years. Geneticists recognize that virtually every individual carries a small number of deleterious or lethal genes, only a few of which can be detected by current methods. In the absence of suitable and parallel educational programs, will such screening procedures be counterproductive by causing unnecessary stigma or fears in carriers?

Difficult questions (for example, the rights of the unborn fetus) have not been adequately resolved. Resolution will require both a more informed public and additional research. An interdisciplinary approach by geneticists, lawyers, sociologists and theologians is desirable, since no single discipline can adequately grapple with the complex issues involved. The Institute is beginning to fund some conferences, and a limited number of research projects in this area. An assessment of current genetic technology may be overdue. The aim is to define the options and their possible consequences. Such research is not expected to prescribe legislation or policy, but rather, to make available for public scrutiny and to policy makers the relevant issues and facts.

C. Research

1. Grants: The largest component of NIGMS support in genetics, utilizing about 80% of total funds, is the research grants program. Individual project grants, fund a wide variety of research ranging from the chemistry of nucleic acids to the genetic analysis of human and animal populations. The broad scope is not surprising, since a wide range of disciplines are expected to contribute to solution of genetic problems. Two major programs, "Genetics" and "Cell Structure and Function", now represent the bulk of NIGMS research support in the basic biomedical sciences.

Increased emphasis on interdisciplinary research has focused attention on larger support instruments, such as large individual project grants and program grants. Applications for such grants are encouraged only if the need for a strongly interactive approach requiring the combined effort of several senior investigators is clearly indicated. They may be the most logical form of support when such an effort requires shared resources that cannot be justified for any one individual investigator. However, a coherent, focused research plan must be demonstrated. We are discontinuing support for some program-projects funded in the past, which we feel do not adhere to the current, more stringent criteria.

2. Genetic Centers: Although individual project grants remain the mainstay of NIGMS support in genetics, the initiation of several substantial "center" grants in genetics takes cognizance of the current increase in emphasis on human and clinical genetics. It is particularly in this area that the cooperation and integration of a variety of skills and disciplines will be required. These centers are conceived as supporting or creating facilities to accomplish work not otherwise possible, and to translate basic advances into clinical application. The centers include both basic and clinical investigations as each relate to overall goals. Prerequisites for such centers include evidence of strong interactions between disciplines and departments, defined research objectives, and strong scientific leadership. (See also the Director's report).

It is confidently expected that most centers will strengthen the training opportunities in the involved departments even though no formal provision is made for training in the awards. Congress itself recognized this potential:

> "It would be prudent to establish genetics research and training centers at major medical schools not only to conduct laboratory and clinical research but to train various types of specialists needed for the application of genetic knowledge to the neglected problems of patients and families suffering from hereditary diseases".
>
> (House Subcommittee report on NIH appropriations, FY 72, p. 34)

During FY 72, the NIGMS actively encouraged the submission of center grant applications in genetics. Eleven new applications and a supplemental application were reviewed. In addition to two previously approved and funded program projects which met all of the criteria for such centers, five new centers were funded. Total funds utilized in FY 72 for this purpose were $4,997,581. The centers are located within medical schools in the states of California (2), New York (2), Texas, Washington, and Maryland. Additional applications will be reviewed and possibly funded during FY 73.

3. Contracts

a. "A Cell Bank of Human Mutated Tissue Culture Cells" ($202,796), The Institute for Medical Research, Camden, New Jersey

In keeping with this Institute's concern to foster maximum progress in human genetics a growing need for a centralized "library" of human tissue culture cells was identified. During FY 72, a number of institutions potentially able and interested in undertaking this task were screened, and the Institute for Medical Research was chosen as uniquely qualified.

Such a "library" is expected to be a unique national resource. Progress will be continually reviewed by staff, with the aid of an expert group of consultants. The facility will develop, grow, and ship to qualified users, cells representative of the many varieties of human genetic disease amenable to study and diagnosis in tissue culture. The availability of such a service is expected to markedly facilitate research, modern clinical practice, and teaching in the area of human genetics.

b. "Biological Macromolecular Separations Technology" ($331,000, 14 mos.) AEC-NIGMS Interagency Agreement. Research performed at Oak Ridge National Laboratory.

The contract was funded to aid in correcting a technological bottleneck in biological research - slow progress in the technology of purifying large molecules of biological interest, particularly nucleic acids. Up to 1970-71, much of the work centered on improvements in separating and purifying bacterial transfer ribonucleic acid (tRNA) species, key components in protein synthesis, and of great interest to the physical chemist as the simplest type of nucleic acid with a well-defined function.

Through this contract, successful methodology was developed for tRNA purification. These techniques are now widely used in many research laboratories, and have been widely quoted in the literature. An important by-product was the availability of the compounds themselves, distributed through this Institute to qualified investigators, and on which much published research has since been based.

More recently, the contractor has shifted attention to the purification of tRNA species of animal origin, a class of materials with additional and separate problems of purification. Attempts are being made to extend the methodology to the purification of larger and more complex nucleic acids.

Discussions are underway between the NIH and the AEC regarding mechanisms which may permit funding of ORNL by grants and through the normal grant review procedures. A research grant proposal dealing with this project has been submitted.

c. "Synthesis of Oligonucleotides" ($99,006), Collaborative Research, Inc., Waltham, Massachusetts

The purpose of this contract is to achieve improved methods for the synthesis of large sequences of nucleic acids. Better methods will be of great importance to the development of molecular genetics, and very few chemists, in relation to the importance of the problems, are working in this field.

The contractor has developed new blocking groups for the synthesis of sequence-specific oligonucleotides. These can be removed specifically and under mild conditions by a proteolytic enzyme. Currently, the methods are being tested by attempted synthesis of a large nucleic acid fragment. In addition, the contractor is beginning to develop methods for chemically joining large nucleic acid fragments to form larger sequences. During FY 73, this contract was renewed at a reduced level. We consider this a self-limiting contract which will be terminated when its defined objectives have been met.

D. Manpower

A recent report on the NIGMS training programs stressed that there were some disciplines and specialties, including human genetics, "in which manpower needs for the next decade are considerably in excess of the supply available, a situation which is due primarily to a paucity of qualified training sites and the difficulty of recruiting qualified trainees."

1. Training Grants: The paucity of qualified training sites is reflected in the distribution of NIGMS training grants in genetics among medical schools and graduate schools. Of some 32 training programs supported in FY 72, more than half were in graduate schools, about a quarter were interdisciplinary programs between graduate schools and medical schools, and less than a quarter were solely in medical schools. However, there are indications that a larger proportion of geneticists, in the future, will be trained in medical schools, and enter the fields of human and clinical genetics. Several factors account for this: (1) The field has become more attractive and exciting because of increased knowledge about the molecular basis of genetic disease. The ability to study human genetic disease in tissue culture or in blood, has made the area more accessible to investigators without clinical training. (2) Because of recent advances, human genetics, particularly of disease, is receiving greater stress in the genetics curriculum. This is evident within the genetics training programs of this Institute. (3) Medical schools are actively recruiting for additional medical geneticists. (4) Genetics centers funded by this Institute should provide a better environment for training in human genetics. In turn, it is expected that such centers will submit training grant applications that can compete successfully with less human-oriented departments.

Some 1200 predoctoral candidates, and about 400 postdoctoral trainees have benefitted from the 30-40 training programs in genetics that have been funded annually by the NIGMS since 1961. A retrospective study of the careers of genetics trainees has shown the value of this program. Among 1,174 predoctoral trainees since 1958, 510 have received the Ph.D. degree and the bulk of another 268 is expected to graduate; 182 are now on university faculties, 54 are on medical school faculties, and others are employed in government, hospital, or industrial positions. Similarly, of 396 postdoctoral trainees (mainly Ph.D.'s, some M.D.'s) 45 are still in training, but 119 are on university faculties, and another 84 are associated with medical schools. The bulk of the remainder are, as above, employed in government, hospitals or industry.

2. Fellowships: Genetics including genetic chemistry, is one of the two largest program areas within the Research Fellowships Branch (the other is cell structure and function). In fiscal year 72 there were 370 awards in genetics (29 percent of the number) at a cost of $4.7 million (30 percent of the funds).

Within the regular fellowships programs, there were 94 predoctoral, 111 postdoctoral, and 31 special fellowships, making up 31, 28, and 17 percent of the total awards, respectively. A particular effort has been made to expand the representation of genetics within the special fellowship group, and the number of awards has increased from 18 to 31 since last year. Human genetics, as yet, accounts for only a small fraction of the three programs. In 1972 there were three postdoctoral and three special fellows falling into this category.

3. Research Career Program: Of the 70 competing Research Career Development awards funded during fiscal year 72, 28 were in the genetics program area, bringing the total number of awards in this program to 122, or 35 percent of the total. Of particular importance is the area of human genetics where three new awards were made in 1972 to bring the total to five. Two of these new awardees had previously been awarded special fellowships (one by NIGMS). The combination of special fellowship research training, followed immediately or several years later by an RCDA, offers an especially effective means of increasing the number of highly qualified human geneticists.

The quality of RCDA recipients in genetics is indicated by the fact that in the last five years five awardees have received either the Eli Lilly Award in Microbiology or the American Chemical Society Award in Biochemistry. These include Dr. David Schlessinger of Washington University (St. Louis) who received his last year of RCDA support out of 1971 funds but was an awardee during FY 72. The other recipients are Drs. Jonathan Beckwith, Mario Capecchi, and Charles Richardson, all of Harvard University, and Dr. Bruce Alberts of Princeton University.

A summary of the fellowships program in genetics for fiscal year 72 follows:

Type of Award	No.	Amount*
Predoctoral	94	$ 420
Postdoctoral	111	841
Special	31	411
Development	122	2,676
Career	12	359
Total	370	$4,707

*In Thousands of Dollars

E. Research Highlights

General: Genetics is a field that advances on a broad front. It is difficult, in any one year, to pick specific "break-throughs" among the more than 400 research grants supported by this Institute. These would, in any case, present a false picture of actual progress, since insights into broad biological problems are usually generated through the complementation of a number of efforts. We have chosen to describe the general progress made in certain areas, with specific examples only as appropriate.

Much of this report stresses human genetics. Last year's effort was largely devoted to strengthening this aspect. The bulk of our research funds continues to support efforts with lower organisms that must be considered as potential models for human genetics. In this section, therefore, we attempt to demonstrate the utility of model systems as "concept factories" and for testing new techniques ultimately applicable to human genetics and genetic disease.

DNA Replication and Repair Synthesis: The biochemistry of DNA duplication remains almost as great a mystery as it was in the fifties, when Kornberg (GM 07581) isolated an enzyme, (now called DNA polymerase I) which appeared to mimic in the test tube many of the properties of DNA synthesis of the bacterium E. coli. The discovery that bacterial mutants exist in which this enzyme is missing, but which divide and grow normally has thrown the field open to investigations seeking to find a "true DNA duplicase". It appears that the DNA polymerase described earlier was an enzyme that permitted the bacteria to repair DNA.

Direct physical evidence for a repair process was first obtained in 1963, in Dr. Phillip Hanawalt's laboratory at Stanford University (GM 09901). This so-called excision-repair process involves the recognition of a damaged region of genetic material (i.e. in DNA), the enzymatic excision of this region, and its resynthesis by repair replication. The process has been demonstrated in a variety of cell types, from the simplest living cells, the mycoplasmas, to human cells in tissue culture. A direct relationship between DNA repair processes and malignancy has been demonstrated in the case of the hereditary disease, xeroderma pigmentosum.

Patients with this defect are unable to repair damaged DNA. They are extremely sensitive to sunlight and develop skin carcinomas and melanomas on prolonged exposure.

At least two grantees of the NIGMS, Dr. Hanawalt, and Dr. Lawrence Grossman (GM 15881) are continuing studies on repair replication. Dr. Grossman's group has isolated five separate enzymes from bacteria which, acting in sequence, excise and then repair a portion of DNA damaged by ultraviolet light. Dr. Hanawalt's group has shown that some of the systems active in repair are also required for the important genetic process of recombination.

Dr. Kornberg's group continues to investigate the mechanism of true DNA replication. It seems highly likely from his studies that at least some ribonucleic acid synthesis must occur to initiate DNA replication, possibly by forming a primer RNA that serves as a site for a covalent attachment of the deoxyribonucleotide that starts a new DNA chain. Since RNA synthesis appears to be subject to a number of cellular control mechanisms, cell divisions may thus also be controlled, in part, through such constraints acting on the initiation of DNA synthesis.

The electron microscope is the primary tool for other studies (Gilbert, GM 17088) which illustrate the great diversity of DNA replication in forms as simple as bacterial viruses. The replicative form of the chromosome of several viruses is a circle, probably involving in different organisms, at least two discrete mechanisms for its formation. Y-shaped rods, formed by yet a third mechanism, are found during the replication of other bacteriophages.

This variety of mechanisms already found in the replication of the very simple chromosomes of lower forms of life would argue that the replication of the very complex chromosomes of mammals and man may be extremely intricate. Here, the research emphasis is still primarily on the chemistry of the components. For example, Dr. Vincent Allfrey's group at Rockefeller University (GM 17383) is investigating the various histones and nuclear phosphoproteins, known to be associated with DNA in the cell nucleus. These proteins can be modified by acetylation, methylation, or phosphorylation, and thus affect the structure and function of chromosomes in a dynamic fashion. A major effort has been concerned with histone changes during the early events in carcinogenesis. It was recently shown, for example, that a major early effect of the carcinogen aflatoxin on liver cells is a rapid loss of the acetyl group of histones.

In summary, the replication of genetic material continues to be an active research problem. The promise of an early and simple solution did not materialize, but much progress has been made. Many catalytic proteins and several independent systems are involved, even in simple organisms. Some of these are involved in net synthesis prior to cell division. Others are implicated in the repair of damaged DNA. The mechanisms for DNA replication appear to differ even among lower organisms. The more complex problem of the replication of mammalian chromosomes is still

primarily studied at the level of chemistry of the components, rather than the mechanisms of the process. NIGMS grantees have taken an active part in all phases of these developments.

The Physical Chemistry of Genetic Material:

It is now some twenty years since Watson and Crick developed the concept that DNA is a double helix of two complementary DNA strands, a structure inherently capable of self-replication. About a decade later, the convergence of several additional concepts began to forge a sound basis for our present ideas on the expression of genetic material: (1) That the first step in the expression of DNA as protein is the transcription of DNA into a complementary RNA. The latter material is the "messenger" of genetic information from the nucleus to the actual sites of protein synthesis. (2) That these sites are organelles in the cytoplasm (the ribosomes) where translation from the nucleic acid code into the polypeptide chains of proteins occurs. (3) That each of the letters of this code, specifying any one of the twenty amino acids found in protein, is a sequence of three nucleotides; this genetic code is now known (for example, the nucleotide sequence GUC specifies the amino acid valine) and appear to be universally valid for all organisms so far studied. (4) That the rates of transcription and possibly also translation are under the constraints of regulation imposed both by the organism's environment and its developmental program. These systems of regulation are also genetically controlled and also involve the transcription and translation of DNA.

As a result of such conceptual developments and aided by greatly increased technological sophistication (e.g., in enzymology, electron microscopy, and physical chemistry), genetics has now moved out of the era in which genetic events and mechanisms could be surmised only by a comparison of parents and offspring. At least in simple organisms, genetic events and processes can now begin to be correlated with the chemical and physical properties of the corresponding DNA. Last year, an NIGMS grantee (Beckwith, GM 13017) isolated, in pure form, a DNA sequence corresponding to a bacterial gene. DNA, specifying a complex ribonucleic acid molecule (a transfer RNA species), has been synthesized by a combination of chemical and enzymatic methods (Khorana). A former NIGMS grantee (Walter Fiers, Ghent, Belgium) has determined the nucleotide sequence coding for the production of the protein coat of a bacterial virus.

The elegance and potential of work done by NIGMS grantees are illustrated by four examples which particularly benefit from interdisciplinary interactions of genetics with physical chemistry and electron microscopy. Other interactions also occur, such as with organic chemistry, pharmacology, etc. The examples point out several generalizations:

(a) Detailed analyses are, at this time, possible only in very simple systems, such as viruses and bacteria. However, techniques of the type described have clear relevance to human genetics and may be applied when certain scientific and technological bottlenecks have

been overcome. For example, details of the structure of mammalian chromosomes have been elusive. Clarification of this might be considerably furthered by techniques for bulk isolation of each of the 23 pairs of human chromosomes; this task has occupied the attention of several of our grantees.

(b) The successes so far have required coordinated efforts in a number of disciplines, including microbial genetics, enzymology, physical chemistry, and electron microscopy.

Drug Resistance in Bacteria (Dr. Robert H. Rownd, University of Wisconsin, GM 14398)

The phenomenon of extrachromosomal (or cytoplasmic) inheritance depends on the fact that non-chromosomal DNA (as in mitochondria, and in plant chloroplasts) is replicated and expressed independently. In bacteria, such extrachromosomal elements, called episomes, also exist. They can be transferred by contact between different bacteria, and even different bacterial species. When separated from chromosomal DNA by buoyant density centrifugation, and examined by electron microscopy, they are seen to be minute, circular DNA molecules.

In certain organisms, episomes are formed which confer resistance to a number of antibiotics (drug-resistance factors or R-factors). The indiscriminate use of antibiotics in medicine and as animal feed additives has led to such R-factors becoming extremely widespread. Since they can be transferred from benign bacteria to pathogens, they pose an obvious threat to the continued effectiveness of antibiotics in chemotherapy. Such R-factors are DNA molecules made up of two components: an r-determinant which harbors the actual genes conferring resistance, and a transfer factor (RTF) thought to be necessary for replication of R-factors and for transferring them from organism to organism.

Dr. Robert Rownd has been studying the effect of culturing the bacterium Proteus mirabilis in the presence of drugs to which it is, due to its R-factors, somewhat resistant. His experiments have shown that the r-determinant can readily keep on copying itself even in concentration of drugs that prevent the bacterium from growing, until enough has been built up to allow the bacteria to break through the drug inhibition barrier.

The density of the chromosomal DNA differs from that of the R factor, in Proteus mirabilis, and thus these molecular species can be separated in the ultracentrifuge on the basis of buoyant density. When these bacteria were exposed to antibiotics, Rownd noted the appearance of a new DNA peak, with a slightly higher density than normal R-factor. With increased length of drug exposure, this peak became larger, until it represented a substantial proportion of bacterial DNA. As the size of this peak increased, the bacteria became more drug resistant.

Rownd has evidence that this new DNA species represents a class of up to 20 linked r-determinants, and that this increase in the number of copies of drug-resistance genes confers the increased drug resistance. In other situations, he has not found a new molecular species, but rather a mutation within R-factors to a state leading to their faster replication, and thus again to a larger number of copies per cell. If such changes in response to drugs occur in nature, they may seriously limit the effectiveness of chemotherapy in the treatment of bacterial infection.

A Biochemical Method for Inserting New Genetic Information into DNA
(Dr. Paul Berg, Stanford University, GM 13235)

The goal of this work is to develop a method by which new, functionally defined segments of genetic information can be introduced into mammalian cells (See also "Genetic (DNA) Therapy" under Section B, "Trends and Needs in Genetics".)

The mammalian virus SV40 can enter into a stable, heritable, and presumably covalent association with the genetic endowment of a variety of mammalian cells. This can also be effected by naked SV40 DNA, although at lower effeciency. Thus, in principle, SV40 DNA molecules, into which a segment of functionally defined DNA had been covalently integrated, could serve as a vehicle to transport these functional DNA's into the cell. There, they might become incorporated into the genome, and be expressed as a new cell function. With this in mind, biochemical techniques were developed which are generally applicable for joining any two DNA molecules.

Using these sophisticated enzymatic methods, various "hybrid" DNA's were constructed, and well-characterized by physical chemical methods. Of especial interest is a molecule constructed from DNA of the bacteriophage lambda, the galactose metabolizing genes of the bacterium Escherichia coli, and SV40. This, in effect, is a trifunctional reagent that contains the genetic information for the mammalian virus SV40, all of the functions of the bacterial virus lambda required for autonomous replication in its host bacterium, and all of the functions of the E. coli galactose operon. Each of these sets of functions has a wide range of potential uses in studying the molecular biology of SV40 and the mammalian cells with which this virus interacts. Thus, geneticists can now tailor-make information-containing molecules such as DNA not occurring naturally through evolution.

Visualization of DNA Structures by Electron Microscopy: Denaturation Mapping and the Heteroduplex Method

These two new techniques show the imaginative ways in which knowledge of the physical chemistry of DNA, electron microscopy, and the detailed genetics knowledge of lower organisms can be combined to yield further insights.

Denaturation Mapping: (Dr. Ross Inman, University of Wisconsin, GM 14711).

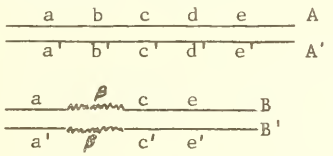
DNA is a double stranded molecule containing four nucleotides, abbreviated A. T. G. and C. The A's of one strand through hydrogen bonding pair with the T's of the other strand, and similarly, C's pair with G's:

```
- A - T - G - C - A - A -
    :   :   :   :   :   :
- T - A - C - G - T - T -
```

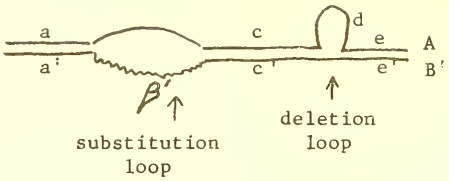
This complementary duplex structure can be disrupted (denatured) by increasing the temperature, or with alkali, resulting in single-stranded DNA. Because of their different mass per unit length, single- and double-stranded DNA can be distinguished from each other in the electron microscope. Furthermore, the bonding between A-T pairs is looser than between G-C pairs. Thus, regions of the DNA molecule rich in A-T pairs can be locally denatured while the integrity of the rest of the DNA is retained. Dr. Ross Inman has taken advantage of these phenomena to prepare "denaturation maps" of simple bacterial viruses. The DNA of a bacterial virus is partially denatured, examined in the electron microscope, and the complex images analyzed by computer. In the bacterial virus lambda, at least 15 discrete regions rich in A-T pairs are seen as "melted puddles" along the DNA strand. Denaturation maps, which are linear representations of the electron microscope image of partially denatured DNA can be used in a variety of ways to help answer biological questions. Any object of interest, such as break points in replication, bound enzyme molecules, positions at which recombination has taken place, and regions containing repeated sequences, can be easily located and studied by the new technique.

The Heterduplex Method (Dr. Norman Davidson, California Institute of Technology, GM 10991)

This method permits one to look for subtle differences in the DNA of closely related organisms. Assume two nucleic acid molecules AA' and BB' derived from such a pair of organisms:



The molecule BB' is derived from a double mutant of the organism AA', with a different region β replacing region b, and with region d deleted. If a mixture of the two molecules is denatured, and then renatured (hybridized), the resultant mixture will contain molecules identical to the original input molecules, and also the heteroduplexes AB' and A'B (AB' is illustrated next page). Regions of non-homology will appear as substitution loops or deletion loops; each are structures readily visualized and differentiated from each other in the electron microscope.



Substitutions or deletions were originally surmised by the more classical methods of genetic analysis. This imaginative new technique now permits a direct comparison between some physical properties (size, position) of a DNA molecule, and its genetic properties.

II. Program Administration

A. Large Interdisciplinary Program, Problems and Opportunities

During FY 72, the Institute has initiated a number of new centers in genetics. This development has focused staff attention on large research grant instruments in general, on the reasons justifying their award, and on their management.

It is generally recognized that such large grants, covering the support for a large number of investigators, have intrinsic disadvantages which must be more than balanced by over-riding potential benefits.

Among the benefits, simplicity in grants management inherent in "lumping", rather than "splitting" has occasionally been cited. This belief has occasionally resulted in the award of large grants which might as readily have been funded by a number of smaller instruments. In reality, large grants are more difficult to administer. Their size allows greater flexibility in the disposition of funds, and thus tends to weaken the influence of the NIH review process. Furthermore, turnover of key scientific personnel is unavoidable in large scientific groups. For such reasons, interim administrative review is often needed. Large grant instruments are difficult to review, since they generally involve a mix of disciplines and scientific components of differing quality.

Recently, problems associated with large grants, and particularly centers, have occupied the attention of a special subcommittee of the National Advisory General Medical Sciences Council. Their report emphasized that the Institute has gone through a period of testing and developing this mode of research support and that the Institute is well-prepared to undertake a defined, practical, and effective center program activity.

What, then, are the valid criteria for making large awards of this type? It is felt that they are desirable only to the extent that they bring about developments in research not possible through other forms of support. These developments include interdepartmental interactions and interdisciplinary collaboration leading toward solutions to

important scientific and medical problems. They also encompass the acquisition of resources, both capital (a facility or major instrument) and human (for example, a needed specialist to complement a group), that cannot be justified by the research efforts of any one investigator.

There is often an untapped potential for valuable collaborative effort, which can be surfaced by the awareness that granting mechanisms exist for its exploitation. Thus, center grants are loosening some traditional departmental structures which, though providing stability, have become needlessly rigid. In other situations, large grants serve to stabilize fruitful collaborative interactions already present, and are a natural instrument of support.

B. Interaction Among Program Components

The Institute's program in Genetics is administered by three branches: Research Grants, Research Training Grants, and Research Fellowships. Traditionally, interactions among these three components have been relatively weak. There are valid reasons for this: The organization of the Research Grants Branch is scientific-problem-oriented. In contrast, the Research Training Branch has necessarily followed the more traditional lines of scientific disciplines, since their support is generally given to departments. For example, an investigator whose research interests deal with nucleic acid biochemistry may have research support from the research grants portion of the NIGMS genetics program. He may also be involved in a training grant in the area of biochemistry, rather than genetics, even though his individual contribution is relevant to the latter area.

The time scale within which the three branches operate is different: The Research Grants Branch deals with scientific development to be exploited during the next few years. In contrast, the Training Grants and Fellowship branches related to over or under-supply of trained personnel in selected specialties over the next decade(s). These different time scales necessitate differing points of view, both overall, and in evaluating individual applications. A case can be made, however, for greater cohesion among the three branches in a programmatic effort such as genetics. The somewhat artificial nature of our support instruments has led to greater administrative separation of responsibilities than may be desirable. Training cannot readily occur in the absence of research; research is an interactive process that implies opportunities for training. Within research grants, graduate students are paid a salary for their research contributions. They are, incidentally, trained. Under training grants, similar individuals receive a stipend toward training and, incidentally, contribute to research. A broader interpretation of our overall objectives, coupled with more flexible support instruments and policy, may help to counteract this somewhat unreal and formalized situation, and would also contribute to the unification of the various programmatic interests of this Institute.

PHARMACOLOGY AND TOXICOLOGY

I. PROGRAM CONTENT

A. Research

The past year has been dramatic and important in the growth and development of pharmacology and toxicology in this country. The Pharmacology-Toxicology Program of this institute, through a national network of centers and program grants, has sustained a high level of productivity in research and research training which is having a significant impact on the delivery of health services and patient care.

Promising new chemotherapeutic substances have been developed for falciparum malaria and schistosomiasis. A new class of organic gallium compounds shows promising antitumor activity. In the genetically determined disease of intermittent acute porphyria, a potentially fatal disease, it has been determined that the primary lesion is a defect in uroporphyrinogen I synthetase. The same enzyme defect was present in erythrocytes forming the basis for detecting the undiagnosed trait in three family members without symptoms, one of whom later had an acute attack. Since drugs and steroids often precipitate an acute attack, the new test serves as an important adjunct to management and genetic counseling. Similarly, a new test on erythrocytes permits diagnosis of slow isoniazid acetylators. Recently, it has been determined that acetazolamide is effective in familial periodic paralysis.

Several studies have shown that poor nutrition, protein depletion, and vitamin C deficiency (GM17184) may increase toxicity to chloramphenicol (GM13606), amphetamines, barbiturates (GM17806), and other drugs. The mechanism is the reduction of liver enzymes (P-450 system) and a greatly decreased rate of drug metabolism (GM16488). Furthermore, the amount of adipose tissue has a marked effect on the pharmacokinetics of water soluble and fat soluble drugs.

New developments in immunopharmacology include new sensitive analytical tests for drugs and metabolites. New animal models for arthritis and synovitis and gout will help in selecting potentially better drugs. Immunosuppressive therapy continues to be favorable in rheumatoid arthritis and psoriasis or psoriatic arthritis. A metabolite of cyclophosphamide is probably the active chemical. In rheumatoid arthritis or progressive systemic sclerosis the urinary excretion of xyloserine has predictive value of a flare up of the disease and is of value in following effectiveness of both old and new drugs.

The treatment of cardiac arrhythmia with lidocaine in the presence of heart failure and liver disease is greatly improved when the altered pharmacokinetics are taken into account.

Advances in toxicology were evident in the recent methylmercury epidemic poisoning in Iraq when an orally administered resin was found beneficial in speeding elimination of the poison from the body. Several investigators are

finding that drugs and chemicals previously thought to be innocuous become potentially toxic to the liver by forming epoxides in minute amounts and forming covalent bonds to enzymes, including P-450. This may also be the mechanism of teratogenesis when the fetus is exposed. Research in perinatal pharmacology has established that essentially all drugs and their metabolites appear in placental, breast milk, and uterine secretions, thus affording opportunity for effects, usually adverse, on the fetus and newborn.

B. Manpower

The last quarter century has witnessed a medical revolution because of the important drugs which have been developed through research. However, the best drug is useless if not prescribed and dangerous if misused. The objective of the NIGMS training grant programs in pharmacology, toxicology, clinical pharmacology, and medicinal chemistry is to eliminate the great shortage of trained investigators in these areas by training scientists who will be capable of confronting problems and developing knowledge relevant to rational pharmacotherapy.

During the past year, the Pharmacology-Toxicology Program Committee reviewed extensively the future needs for research manpower in the above areas. They concluded in their report that "to meet the nation's urgent need, training support in these disciplines should be maintained at its present levels at the very least." Because of the increasing importance of drugs in patient therapy and the need for their sophisticated and effective use, the number of trained individuals in these fields must increase during the next decade. The nation's welfare requires maintenance of ability to deal with drugs and environmental chemicals, and the need for individuals and organizations to fulfill this requirement will increase markedly in the future.

1. Training Grant Programs

The level of support for training grants has been essentially constant for the past several years. For FY 72, as the following table shows, a further small decrease, with 61 active programs at $4.549 million, compared respectively with 66 programs at $4.517 million for FY 71. During the year, 7 renewal and 1 new application were funded.

Active Research Training Grants--FY 1972

Training Area	Active Grants	Amounts Awarded (thousands)	Trainees			Total
			Pre	Post	Spec	
Pharmacology	39	$3,322	363	82	1	446
Clin Pharmacol	5	286	-	9	9	18
Toxicology	8	352	39	4	-	43
Med Chemistry	9	589	87	3	-	90
Total	61	$4,549	489	98	10	597

2. Postdoctoral Fellowships

The fellowships program will soon be entirely postdoctoral, when the current predoctoral awards terminate. Three types of postdoctoral awards are now made; namely, postdoctoral fellowships, special fellowships, and career development awards. The total expenditure for fellowships increased approximately 20 per cent over the previous year, to $1,435 million, despite the phasing out of predoctoral fellowships. The number of awards remained essentially the same, but the expenditure was greater because of the shift from the predoctoral to the more expensive postdoctoral and career development programs. There was a significant increase in career development awards, from 28 to 38.

Research Fellowships in Pharmacology-Toxicology--FY 1972

Type of Award	Pharmacol.		Toxicology		Clinical Pharmacol.		Medicinal Chemistry		T o t a l	
	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.
Predoctoral	21	$ 92	1	$ 5	0	$ 0	6	$ 28	28	$ 125
Postdoctoral	20	156	3	24	0	0	8	56	31	236
Special	10	117	1	15	0	0	1	21	12	153
RCDA	22	489	3	67	3	75	10	200	38	831
Career	3	90	0	0	0	0	0	0	3	90
Total	76	$944	8	$111	3	$ 75	25	$305	112	$1,435

NOTE: Amounts shown in thousands of dollars

The fellowships program provides a mechanism for individuals trained in a variety of areas to obtain postdoctoral training in pharmacology and closely related disciplines at institutions which do not have training grants. This makes it possible to increase the range of training possibilities and takes advantage of special research opportunities which might not otherwise be possible.

3. Career Development Awards

It is interesting to examine the positions now held by former career development awardees. As of July 1, 1972, only 69 of 500 former awardees have worked in the area of pharmacology-toxicology. These 69 have a relationship to the Pharmacology-Toxicology Program by meeting one or more of the following criteria: (a) current position is in pharmacology, (b) the RCDA was in pharmacology, (c) holds membership in ASPET, (d) principal investigator on an NIGMS grant in the pharmacology-toxicology area, and/or (e) both RCDA and current position are in anesthesiology. Anesthesiologists are included in this summary because of their close association with pharmacology. The data are summarized in the following table.

Former Development Awardees in Pharmacology-Toxicology--
Positions as of December 31, 1971

	Chmn & Prof.	Prof.	Assoc. Prof.	Asst. Prof.	Other	Total
Academic position:						
1. In pharmacology department						
--RCDA in pharmacology dept.	8	22	5	-	-	35
--RCDA in other department	3	3	-	-	-	6
2. In other department						
--RCDA in pharmacology dept.	3	4	2	-	-	9
--RCDA and presently in dept. of anesthesiology	2	3	1	1	-	7
--RCDA in other department: member of ASPET and/or PI on NIGMS grant	2	4	1	-	-	7
Non-academic position:						
1. Members of ASPET and RCDA in departments of pharmacology	-	-	-	-	5	5
TOTAL	18	36	9	1	5	69

It is rewarding to find that 36 (88 per cent) of the 41 former awardees who are presently in departments of pharmacology have attained the rank of full professor, and 11 of these are department chairmen. Interestingly, 3 department chairmen who held RCDA's in other departments were previously located in departments of biochemistry. On the other hand, 2 professors held an RCDA in physiology, and a third was located in a department of surgery.

An additional 23 former awardees were included because they held an RCDA in a department of pharmacology or anesthesiology, were ASPET members, or were principal investigators on an NIGMS research grant in the pharmacology-toxicology program area. It is equally rewarding that, of these, 7 are department chairmen, 11 are full professors, 4 are associate professors, and 1 is an assistant professor. Half of this group is presently in departments of physiology or biochemistry, and the others are using biochemical or physiological approaches in their research.

Five former awardees have non-academic positions with the government, industry, or research laboratories. Their job titles suggest predominantly administrative responsibilities. Each of the five is a member of ASPET and held his RCDA in a department of pharmacology.

Three clinical pharmacologists received development awards as of July 1, 1972. Their proposed studies include the effects of intestinal motility on drug absorption, the effect of blood flow on drug intoxication, and pharmacokinetics of several drugs in man.

C. Research Highlights

1. Cytochrome P-450

Ronald W. ESTABROOK, University of Texas P11 GM16488

Mixed-function oxidations catalyzed by cytochrome P-450. Before the clinician can utilize the wealth of information concerning drug and steroid metabolism which has been accumulated during the past decade, we must increase our understanding of the molecular mechanisms of these reactions. To this end, this grantee has greatly extended studies on the molecular mechanisms of mixed-function oxidations catalyzed by cytochrome P-450 in both liver and adrenal cortex. He has characterized an oxygenated form of cytochrome P-450 which is presumably the direct precursor of the actual hydroxylating species. In addition, evidence has accumulated indicating that reduced cytochrome b_5, a pigment present in many tissues, may serve as the donor of the second electron required for overall substrate hydroxylation in liver microsomes. Moreover, rapid kinetic studies revealed that the rate-limiting step in these reactions may be the donation of the second electron to cytochrome P-450. The basic goal is to better predict the rate at which a drug is metabolized. The ability to alter or modify drug response will depend on the ability to regulate the control step in drug degradation.

Studies have also been carried out on the interaction of insecticide synergists with cytochrome P-450 of liver, showing that a relatively non-toxic substance, such as piperonyl butoxide, can inhibit the oxidation of many drugs. This is of significance to the clinician since, through the use of these compounds, lower doses of a drug may be required to maintain a longer duration of action of the desired therapeutic agent. A general principle is now developing from these studies, as well as from studies with the anesthetic, halothane, and the inhibitor, SKF-525A, indicating that the formation of non-functional complexes of metabolic products with cytochrome P-450 alters the rate of metabolism of other drugs.

Gilbert J. MANNERING, University of Minnesota P11 GM15477

Purification and characterization of the components of the hepatic mixed-function oxidase system responsible for drug biotransformation. Solubilization and purification of the hepatic microsomal system responsible for drug metabolism has been difficult because the P-450 hemoprotein component of the system is intimately associated with the microsomal membrane and it is rapidly inactivated when this association is disrupted by the agents commonly used to solubilize membranes. Biochemically active cytochromes P-450 (obtained from untreated or phenobarbital-treated rats) and cytochromes P_1-450 (obtained from 3-methylcholanthrene-treated rats) have been successfully solubilized with fourfold purifications. The reductase required in the system is more readily solubilized than the P-450 cytochromes and this component has also been purified. These studies open the door to eventual purification and characterization of all of the components of the system. The ultimate goal is to define the working parts of the system and answer the question as to how many P-450 hemoproteins exist. These studies have already demonstrated that P-450 and P_1-450 are different chemical entities.

H. George MANDEL, George Washington University PO1 GM13749

Two species of hepatic cytochrome P-450. Mitotane (chemically related to DDT) is an agent with high selectivity for the adrenal cortex. It was previously shown to inhibit the rate-determining step in the biosynthesis of adrenal cortical steroids, and this action apparently forms the basis for its usefulness in treating cancer of the adrenal cortex. Current studies have shown that mitotane induces the hepatic biosynthesis of cytochrome P-450 in the dog and thus promotes the metabolism of many compounds, such as ethylmorphine, aminopyrine, and aniline. The metabolism of pentobarbital, however, is depressed secondarily to the effects of mitotane on the adrenal cortex. This suggests the presence of at least two species of hepatic cytochrome P-450 in the dog, one of which is dependent on the integrity of the adrenal cortex for its maintenance.

2. Factors Influencing Drug Metabolism

Individuals metabolize drugs at widely different rates due to genetic, environmental, or drug interaction factors. Because drug effects are usually terminated by biotransformation, rates of drug metabolism are very pertinent to duration and intensity of action. Rates of metabolism are frequently reflected in blood levels of the drug observed at a selected time after its administration or at regular intervals when the drug is given chronically. Because of recent advances in methodology, it is possible to accurately measure blood levels of many drugs with high degrees of specificity.

Daniel L. AZARNOFF, University of Kansas P11 GM15956

Bioavailability. Bioavailability is also an important factor affecting the response to drugs. Studies with digoxin have indicated that the availability of a drug from different dosage forms does influence the half-life of the drug and can lead to serious misinterpretation of the data.

Thomas R. TEPHLY, University of Iowa P11 GM12675

Species differences and metabolism of glutethimide. Analysis of the plasma of glutethimide-intoxicated humans using gas chromatography showed that, while glutethimide plasma levels were decreasing in the comatose patient, a hydroxylated metabolite of the drug accumulated to high levels in the plasma. At approximately the mid-point of the comatose period, the patients exhibited plasma concentrations of glutethimide usually associated with sedative doses while metabolite levels were very high. The patients usually recovered from the coma when metabolite plasma levels began to decline. The hydroxylated metabolite was not observed in the blood after therapeutic doses of glutethimide to humans or after toxic doses to rats or dogs. The results show that toxic doses of glutethimide are metabolized differently in humans than in two common laboratory species. These species, which are known to metabolize the drug like humans after therapeutic doses, are often used as animal models to study ways to reduce the toxicity of glutethimide. The data suggest that the rat and dog are not adequate animal models in which to study glutethimide intoxication.

Bert N. LA DU, Jr., New York University P01 GM17184

Vitamin C and drug metabolism. The effect of a deficiency of vitamin C on drug metabolism in guinea pigs has been studied. Cytochrome P-450 and P-450 reductase levels are decreased, which seems to be responsible for an associated decrease in drug metabolism in scorbutic animals. Drug metabolism is restored slowly (6 to 10 days are required) after animals are re-fed a diet high in ascorbic acid. Apparently, the recovery of drug metabolism depends upon new protein synthesis, not just repletion of the vitamin store.

Silvio GARATTINI, "Mario Negri," Milan P01 GM18376

Role of adipose tissue in the distribution of drugs. It is remarkable that drugs of a similar structure may be taken up by fat tissue in a different manner. Investigations conducted in vitro and in vivo with two butyrophenones, haloperidol and trifluperidol, have shown that, while the former does not accumulate in adipose tissue, the reverse is true for the latter. Both drugs are widely used in the treatment of psychotic and psychoneurotic conditions. This raises the question, requiring further study, of the need for gauging dosages according to the fat content of the patient's body (trifluperidol) and emphasizes the need for determining all the important parameters for each drug in a given group rather than making extrapolations.

Effect of age on drug metabolism. The influence of age on drug metabolism was followed in premature and full-term infants, and children, who received a single dose of diazepam therapeutically. In premature infants, the level of this drug was higher, while that of N-methyl diazepam was lower than in the other two groups. Hydroxylation of diazepam increases with age but was undetectable in premature infants. It was also found that the anticonvulsant action of diazepam is about 8 times more potent in newborn than in adult rats. These clinical and experimental findings strongly point to the need for revising currently used dosages of tranquilizing agents administered to infants.

In another set of studies, aminopyrine disappeared from the plasma of old subjects at the rate of 9 per cent per hour, as opposed to 17 per cent per hour in young persons.

3. Methodology

Gilbert J. MANNERING, University of Minnesota P11 GM15477

High-speed liquid chromatography. Development of methodology is a necessary, integral function of any successful research program. The use of coupled gas-liquid chromatography-mass spectrometry has found application in a number of studies. This grantee, pioneering in application of high-speed liquid chromatography to pharmacology, is currently using this technique to study rates of metabolism of a selected series of barbiturates as part of a study of the relationship between the rates of metabolism of barbiturates and the ability of these compounds to bind to microsomal protein. He has found that the ability of barbiturates to bind to microsomal proteins is almost exactly the same as their ability to combine with serum proteins.

Thomas R. TEPHLY, University of Iowa P11 GM12675

Prostaglandin radioimmunoassay and localization. Antibodies to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have been used to establish and standardize the radioimmunoassay for this potent compound which may function like a local hormone in many organs. One interest in prostaglandins is to determine how they are involved with intrauterine contraceptive devices and oral contraceptive agents; $PGF_{2\alpha}$ has been proposed as a factor released by the uterus causing regression of the corpus luteum, an event which could prevent or terminate early pregnancy. The assay will be used to determine uterine content and in vitro uterine release of $PGF_{2\alpha}$ and to study effects of anti-inflammatory agents, such as indomethacin, which inhibit prostaglandin synthesis.

4. Teratology

Thomas R. TEPHLY, University of Iowa P11 GM12675

Methylmercury and drugs. The Oakdale Toxicology Center investigated delayed effects of methylmercury, the notorious water pollutant, in young rats born of mothers treated with methylmercury in mid-pregnancy. This situation is somewhat analogous to the methylmercury poisonings which took place in Japan five or six years ago and this winter in Iraq. They found that single administration of methylmercury to pregnant rats resulted in the birth of offspring with a number of defects in several systems of the body.

Previously, they reported studies involving malformations produced in mice by treatment of pregnant animals with diphenylhydantoin, a drug widely used for the control of epilepsy. Studies have pointed out a species difference in response between the mouse and the rat. Subsequently, other workers found malformations in humans which closely resemble those found in the laboratory in mice.

Study of another widely used anticonvulsant drug, diazepam (Valium), has shown similar teratogenic changes in mice to those found with diphenylhydantoin. The center recommends that the human population be carefully surveyed for malformations in newborns of mothers treated with diazepam during early pregnancy. The problem of possible diazepam teratogenicity in humans and the need for epidemiological studies of possible diazepam teratogenicity is considered to be of great importance. The reason for this assessment of the problem is that diazepam is widely used, not only as an anticonvulsant but also as a tranquilizer and sedating drug.

Thomas W. CLARKSON, University of Rochester P11 GM15190

Mercury poisoning. This toxicology center specializes in research on heavy metals. This year, these grantees were invited to the Baghdad Medical Center, from February 24 to March 9, to help with a crisis of mercury poisoning in Iraq. The source of exposure was 80,000 tons of mercury (Granoson M)-treated grain imported from Mexico in October 1971. The purpose of the visit was to give lectures on mercury poisoning to the medical school and to study cases of mercury poisoning.

5. The Epoxide Hypothesis

Marjorie G. HORNING, Baylor College of Medicine P11 GM16216

Harmless to harmful via the epoxide route. Epoxides may be of great importance in producing toxicologic effects. This grantee has found epoxides of allyl-substituted barbiturates in the rat; the chief metabolic pathway for diphenylhydantoin in the human involves an epoxide intermediate. The epoxides of three allylic barbiturates (alphenal, diallylbarbituric acid, and secobarbital) have been identified in rat urine. A method of trapping epoxides as chloro-trimethylsilyloxy derivatives was developed in these studies.

Epoxides may have cytotoxic or mutagenic properties. The possibility must also be considered that epoxides are also teratogenic, in view of the high incidence of physical abnormalities present in infants of mothers maintained on anticonvulsant drugs (diphenylhydantoin, mephobarbital, phenobarbital). These preliminary results suggest that drugs metabolized by way of epoxides should not be administered to pregnant women or to newborn infants if other equally effective drugs are available. For example, pentobarbital could be substituted for secobarbital as a sedative during labor and delivery.

Silvio GARATTINI, "Mario Negri," Milan P01 GM18376

A stable epoxide of carbamazepine. During studies on the pharmacokinetics of orally administered carbamazepine in rats and in man, a new metabolite, carbamazepine-10,11 epoxide, was isolated and identified by means of TLC, UV, and GLC analyses. The identification of a stable epoxide as one of the major metabolites of carbamazepine is of interest because it may be a precursor of hydroxylated metabolites, now being identified, and because this metabolite may be involved in the cases of agranulocytosis, attributed to carbamazepine.

6. Drug Interactions

Tom S. MIYA, Purdue Research Foundation P01 GM15005

DPH inhibition. Diphenylhydantoin (DPH) was shown to block the ability of tolbutamide to lower blood sugar. In addition, DPH also blocks the diabetogenic action of the pancreatic beta cell cytotoxic agent, alloxan. It is possible that DPH exerts its action by protecting the beta cell binding site(s) against tolbutamide and alloxan. Preliminary findings were published in Diabetes 21, 80-83 (1972). This phenomenon also has relevance to cancer chemotherapy, since alloxan and related compounds may be useful for the treatment of pancreatic tumors if a suitable means of blocking the diabetogenic action can be found.

Marjorie G. HORNING, Baylor College of Medicine P11 GM16216

Maternal-fetal drug transfer. There is no "placental barrier" which protects the fetus from drugs administered to the mother. As a consequence, the fetus is exposed to adult concentrations of active compounds administered to the mother. In addition, the drugs and/or metabolites are stored in fetal tissues, and after delivery the newborn infant must dispose of these foreign compounds. In contrast to other mammals (guinea pigs, rabbits, and mice), the human neonate is able to metabolize some drugs from the day of birth. The microsomal enzymes necessary for aliphatic, allylic, and aromatic hydroxylation are operative from day one. Some enzyme systems are considerably less active in neonatal tissues than in the maternal tissues, for example, enzymes involved in the demethylation of caffeine and related xanthines. Following caffeine ingestion, the major xanthines in maternal urine are the monomethyl xanthines. After placental transfer of caffeine, the major xanthine in neonatal urine is caffeine. The neonate can also carry out conjugation of drug metabolites with glucuronic acid, indicating an active UDP-glucuronyl transferase system.

Drugs administered to the mother have been identified in breast milk. Depending on the concentration in milk, appreciable quantities of drugs could be ingested by the breast-fed infant. One mother was being treated with daily doses of an anti-convulsant drug, Zarontin (ethosuximide), which was identified in breast milk for the duration of breast feeding (5 weeks). It would seem advisable that physicians be alerted to this route of drug ingestion by the neonate and each drug be judged individually as to its potential effect on the nursing infant.

Thomas C. BUTLER, University of North Carolina P11 GM13606

Penicillin hypersensitivity. Allergy to the penicillins accounts for about half of all drug hypersensitivity reactions and constitutes one of the most serious problems in therapeutics. This work contributes to an understanding of this important phenomenon and to the possibility of producing a drug having the same antibacterial action as the penicillins but not their allergenic potential.

It had been proposed that penicillenic acid was the intermediate that reacted with protein to produce allergenic proteins. The formation of benzylpenicillenic acid has been reinvestigated by studying the kinetics of appearance and disappearance of 320 nm absorbancy. Benzylpenicillin in solution slowly rearranges to yield an equilibrium mixture of benzylpenicillenic acid and its thiazolidinyl-oxazolone isomer, the latter being the reactive intermediate responsible for the introduction of the haptenic penicilloyl group into the patient's protein to produce allergenic protein. The 320 nm absorption observed in benzylpenicillin solutions is probably produced chiefly by S-oxidized derivatives of benzylpenicillenic acid and by penicilloylpenicillenic acid, both of which are chemically unreactive.

D. Contracts

The Pharmacology-Toxicology Program has used contracts to accomplish a variety of objectives as the need has arisen. Contracts are negotiated when this is the most appropriate mechanism of support. Currently, the program supports 26 projects under 8 research contracts. The accomplishments of some of these contractors who received new funds in FY 72 are described below.

1. Evan C. HORNING, Baylor College of Medicine NIH 69-2161

In the past year this contractor completed initial redesign of a gas chromatograph-mass spectrometer for use in biomedical research. A contractor-subcontractor team developed a new methyl ester of amphotericin B. It is water soluble, in contrast to amphotericin B; seems less toxic in animal studies; and appears effective against fungal disease and possibly malaria. When insoluble polyene macrolides were given orally, fecal extraction of sterols increased and the composition of fecal sterols changed, with more cholesterol present. This preliminary but exciting work may relate to atherosclerotic disease in man.

2. Hershel JICK/Dennis SLONE, Boston University PH 43-67-1485

The Boston Collaborative Drug Surveillance Program is supported by this contract. Recently, the contractor found that there is an interaction between chloral hydrate and warfarin. Those receiving continuous chloral hydrate therapy required significantly less warfarin during the induction phase for anticoagulation than those receiving none. Thus, interaction between these drugs is of clinical importance, and care should be exercised when they are administered concurrently.

The contractor has studied the relationship between digoxin arrhythmias and ABO blood groups. Of 1,080 patients who received digoxin and were consecutively monitored, arrhythmias attributed to the drug were found in 144, or 13.3%. The proportion of arrhythmias among 510 patients with blood group O was 10%, while that for the 570 patients of A, B, and AB (non O) was 16.3%.

A statistically significant association between tetracycline administration in patients receiving diuretics and the development of clinically significant rises in blood urea nitrogen (BUN) levels has been found. They concluded that tetracycline should be avoided in patients who were also receiving diuretics when alternative antibiotics could be substituted.

Surveillance of 240 medical inpatients receiving prednisone revealed a correlation between the frequency of side effects, the mean daily prednisone dose, and the serum albumin. When serum albumin concentration is less than 2.5 g per 100 ml, the frequency of prednisone side effects is doubled. The binding of prednisolone, the active prednisone metabolite, to albumin and corticosteroid-binding globulin was measured. It was clearly shown that hypoalbuminemia can result in increased free prednisolone levels, which partly explains the increased frequency of side effects in such patients.

3. Monroe WALL, Research Triangle Institute PH 43-65-1047

This contractor has almost completely mapped out the metabolism of norethynodrel in the mouse, rat, and man. Norethynodrel is one of the most widely used oral contraceptives. In preliminary studies, RTI has shown that the metabolism of chlormadinone in the beagle dog differs from metabolism of the drug in man and rodents. This is highly significant in that this drug was removed from the market because of the production of mammary tumors in the beagle; further work is necessary to determine the significance to man.

Using high pressure liquid chromatography, RTI has developed a new assay for alpha-d-propoxyphene blood concentrations. An accurate, sensitive method had not been available. Over 600,000,000 capsules of this analgesic drug are sold in the United States annually, and there have been serious side effects, including death, from misuse of the drug. The inability to determine blood concentrations hindered the rational treatment of overdose.

RTI has also developed techniques for labeling commonly used drugs with non-radioactive carbon 13. They are at the forefront in developing methodology for the determination of low levels of carbon 13 in body fluids.

Under an interagency agreement, the Atomic Energy Commission synthesizes and provides simple chemical precursors, labeled with carbon 13 (e. g., 13C-acetic acid). These labeled precursors are used by institute grantees or contractors to synthesize more complex molecules. These are often commonly used drugs used for tracer studies in clinical situations where drugs labeled with the radioactive carbon 14 cannot be used.

4. National Academy of Sciences/National Research Council PH43-65-44
Task Order No. 5

This contract supports the Drug Research Board (DRB). This represents a truly outside source of advice, since the Institute does not influence the selection of its members. It provides a forum where representatives of other government agencies, industry, and the academic-scientific community can communicate and advise those who sponsor drug research or who develop, prescribe, or regulate drugs.

The DRB Committee on Problems of Drug Safety has been instrumental in the planning and managing of four program-relevant workshops and conferences during the past year.

This contractor has also been responsible for numerous and important reports and publications related to the objectives of the Pharmacology-Toxicology Program.

II. PROGRAM ADMINISTRATION

A. Program Support

The program support for fiscal year 1972 has increased to $18.4 million, an increase in research grants of approximately $1.3 million over fiscal year 1971. The Congressional line item for support of centers increased to $5.593 million as compared to $3.593 in FY 1971. Funding of training grants has been fairly constant for several years, with $4,549 million in FY 72. The total expenditure for fellowships increased about 20 per cent in FY 72 to $1.435 million, mainly due to the shift to postdoctoral programs. The career development awards increased significantly, from 28 to 38. The intramural Pharmacology Research Associate Training (PRAT) Program increased from 15 to 18 stipends, to $336,000. Only 5 of the 8 active contracts received new funds in FY 72, in the total amount of $1.962 million.

B. Centers

The major research thrust continues to be in support of 11 centers and 11 program projects and approximately 40 regular project grants. Two-thirds of these programs have been critically reviewed, and renewal applications for all center and all but 4 program projects have been approved for funding. One new center and one new program project application have also been approved for funding.

All 22 of the PT centers and program projects are university based except one. Of the university-based grants, all are associated with medical schools except two. By far the majority of the grants are administratively based in or strongly associated with departments of pharmacology and toxicology. The primary orientation is in the broad field of pharmacology, although appreciable attention is devoted to toxicology and two of the centers are concerned primarily with toxicology.

Most of the PT centers have acquired distinctive physical facilities, usually within or contiguous to a department of pharmacology. Some have achieved a separate building for at least part of their activities, notably the Upjohn Center at Michigan, the Oakdale Toxicology Center at Iowa, and the Clinical Pharmacology Center at Kansas.

A majority of the centers have developed strong ties with clinical departments. This has been most effective in those centers headed by clinical pharmacologists. The associations are most commonly with the department of medicine but also include departments of pediatrics, psychiatry, anesthesiology, and others. All of the program project grants have adequate physical facilities. About three fourths of these grantees have also formed strong associations with clinical departments.

The program continues to influence the course of pharmacology in this country. Pharmacologists trained in one center move to head up other centers or assume key positions in pharmacology and toxicology. Another trend is for directors of clinical pharmacology divisions to head up departments of pharmacology. This has occurred in at least three departments in the past year.

C. Communications

The PT Program has persistently sought methods to close the gap between the discovery of new knowledge related to rational pharmacotherapy and its application at the bedside. Through the direct efforts of its staff and the Pharmacology-Toxicology Program Committee, the Institute has made some progress. A rather broad program in communications has been directed to the scientific community through books, publications, and conferences, cosponsored in part under the NAS/NRC Drug Research Board and the Committee on Problems of Drug Safety.

Reports of the International Conference on Adverse Reactions Reporting Systems and the Conference on Clinical Pharmacology were published by the National Academy of Sciences in the fall of 1971. The Report on Adverse Reactions Reporting Systems recommended the establishment of a national center for drug surveillance.

Three workshops took place in 1971 which will lead to similar publications. These were the Conference on Pediatric Clinical Pharmacology, November 8-9, Washington, D. C.; the Workshop on Immunopharmacology, November 15-19, New York University; and the Conference on Bioavailability, November 22-23, co-sponsored by the Drug Research Board and the United States Pharmacopoeia, Washington, D. C.

To facilitate international communication, plans are under way to establish, through the NIH Fogarty International Center, the Continuing Conference on Clinical Pharmacology and Drug Metabolism, between Sweden and the United States, under the auspices of NIH-NIGMS and the Swedish Medical Council. A small workshop would be held each year in alternate countries.

RESEARCH GRANTS BRANCH

During FY 72 there were a number of changes in professional staff in the Research Grants Branch. Dr. Emilie Black was named Acting Assistant Branch Chief for Clinical Programs and Chief of the Clinical Sciences Section replacing Dr. Larry Rose who resigned. Dr. Fred Bergmann was named Chief of the Genetics Section, which was expanded, and Dr. Vincent Price as Chief of the Cell Structure and Function Section and Director of the Cellular Basis of Disease Program. Dr. Raymond Bahor assumed the responsibility of administering the research grants as well as the training grants in the Pharmacology/ Toxicology Program. Two additions to the staff were Dr. William Gartland, Genetics, and Dr. Matti Al-Aish, Cell Structure and Function.

In spite of the fact that the procedure of negotiating reductions of non-competing continuation grants was discontinued, the Institute was able to fund 59% of approved applications compared with 42% in FY 71 and only 19% in FY 70. A total of 512 applications was funded including 15 supplements. Forty percent of the paid competing applications were renewals of ongoing projects while 57% were new projects.

Table 1 shows the distribution of grants and funds in the various programs. The total of 1234 grants includes 41 ongoing genetics grants which were funded by the NIGMS but which are in the programs of other Institutes. The number of awards for this Institute increased by 84. The balance between programs was essentially the same as the previous year.

The distribution of grants by class and their average size is shown in Table 2. Regular projects account for 92% of awards and 61% of the dollars, whereas 8% of the awards (program projects and centers) consume 39% of the research funds. In spite of the initiation of the center program in genetics, this ratio is similar to that of the previous year.

Special mention should be made of the excellent job done by the members of the Genetics Section in programming and establishing the new Genetics Centers. This activity was very time-consuming and the results reflect their effort. The cooperation and special assistance of the Division of Research Grants was a real help and is greatly appreciated.

The Research Grants Operations Unit did a very commendable job in coping with the tremendous workload and the many problems resulting from the institution of centralized payment of indirect costs by the OFM. The new system has, so far, been quite unsatisfactory; it has also more than doubled the accounting workload.

The authorization for the General Medical Program Project Committee expired at the end of the fiscal year. In keeping with the desire of the Administration to reduce the number of committees, the GMP Committee was allowed to lapse since other means of accomplishing their function presently exist.

TABLE 1
Program Summary--Research Grants Branch

Program	Active Grants As of 6/30/72 No.	Active Grants As of 6/30/72 Amount	Awards from Fiscal Year 1972 No.	Awards from Fiscal Year 1972 Amount
Pharmacology/Toxicology	71	$ 10,893,500	61	$10,122,381
Engineering in Biol. and Med.				
A. Biomed. Engineering	47	6,277,369	41	5,872,574
B. Auto. of the Clin. Lab.	55	5,126,748	46	4,615,180
Clinical Medicine				
A. Anesthesiology	18	4,727,058	15	4,321,361
B. Diagnostic Radiology	23	2,889,939	19	2,761,250
C. Trauma	33	4,047,348	30	3,423,562
D. Clinical Medicine	12	933,242	12	928,092
Fundamental Sciences				
A. Genetics	270	23,324,932	246	20,980,323
B. Genetic Chemistry	191	11,450,408	168	9,654,518
C. Cell Structure & Function				
1. Cell Structure	171	9,712,359	150	8,849,925
2. Cell Function				
a. Level of the Whole Cell	84	4,586,732	71	3,770,263
b. Subcell. Level	189	10,548,694	181	10,169,659
D. Bio-rel. Chem. Proc.	159	6,827,156	139	6,038,229
E. Adaptation & Behavior	16	1,836,400	14	1,576,204
Subtotal	1,339	103,181,885	1,193	93,083,521
Other Institutes (Genetics)	42	1,493,741	41	1,448,479
Grand Total	1,381	$104,675,626	1,234	$94,532,000

TABLE 2
Distribution of Grants by Class FY 72

Program Area	Regular Projects		Program Projects		Centers	
	No.	Amount	No.	Amount	No.	Amount
Pharmacology/Toxicology	40	$ 1,794,931	9	$ 2,078,837	12	$ 6,248,613
Biomedical Engineering	30	1,933,816	11	3,938,758	-	- -
Automated Clinical Laboratories	41	2,877,160	5	1,738,020	-	- -
Anesthesiology	9	278,001	2	896,063	4	3,147,297
Diagnostic Radiology	14	526,233	3	1,145,772	2	1,089,255
Trauma	22	1,111,847	-	- -	8	2,311,715
Clinical Medicine	10	507,666	2	420,426	-	- -
Genetics	227	10,959,833	12	5,023,128	7	4,997,362
Genetic Chemistry	167	9,331,314	1	323,204	-	- -
Cell Structure	148	8,575,715	2	274,210	-	- -
Level of Whole Cell	68	3,147,907	3	622,356	-	- -
Subcellular Level	178	8,867,196	3	1,302,463	-	- -
Bio-related Chemical Processes	139	6,038,229	-	- -	-	- -
Adaptation & Behavior	12	935,940	2	640,264	-	- -
Totals:	1,105	$56,885,778	55	$18,403,501	33	$17,794,242
% of all awards:	92%	61%	5%	20%	3%	19%

RESEARCH TRAINING GRANTS BRANCH AND FELLOWSHIPS BRANCH

Research Manpower Study

During FY 72, there were continuing efforts by the Research Training Grants Branch and the Research Fellowships Branch to evaluate the supply and demand for research-trained manpower in the various program areas for which the NIGMS has responsibility. A summary report was prepared in the fall of 1972 by Institute staff, using its best judgment to reconcile and coordinate the 16 separate research training committee manpower reports. The various program areas were judged to fall into three general groups or categories.

In category I are those program areas in which manpower needs for the next decade considerably exceed the supply available, a situation due primarily to the paucity of qualified training sites and the difficulty in recruiting qualified trainees. This category consists of (a) certain clinical specialties and subspecialties--anesthesiology, diagnostic radiology, clinical pharmacology, and human genetics; (b) three meagerly represented but significant disciplines--nutrition, epidemiology, and biometry; and (c) three interdisciplinary program areas designated medical scientist, multidisciplinary medical, and multidisciplinary biological.

In category II are those disciplines which are not now saturated with regard to the need for scientific manpower, but which may become so in the next decade; these will require careful monitoring to assure that there is no substantial mismatch of the supply with demand. This category includes four of the traditional preclinical disciplines--anatomy, microbiology, pathology, and pharmacology (including toxicology). In this category are also surgery, including trauma, behavioral sciences, clinical chemistry, and engineering in biology and medicine.

In category III are those program areas in which there is current or imminent saturation of the demand for scientific personnel, namely, biochemistry, organic chemistry, medicinal chemistry, biophysical sciences, and physiology.

This summary manpower report was made public in testimony before congressional committees and is serving as the basis for future program and budget planning. It is well recognized that the information on which the individual committee reports were based will need updating from time to time, since the national research manpower supply and demand picture has been shown to change within a surprisingly small number of years. The Nutritional Sciences, Microbiology, Engineering in Biology and Medicine, Behavioral Sciences, and Radiology Training Committees have already published edited versions of their research manpower reports, and the Pathology Training Committee is expected to do so shortly. As part of a larger project, the NIGMS has provided contract support for a study of research contributions by surgeons and departments of surgery, with an analysis of the types of research training which the successful surgical scientists have had. This contract, expected to require two years, is conducted by Dr. Marshall Orloff of the University of California, San Diego.

Program Directors' Meetings

One of the mechanisms for staying in touch with our consultants in the various disciplines is through sponsored meetings of the program directors. Both the Surgery and the Radiology Training Committees held such meetings during 1972. The Surgery program directors met with the Surgery Training Committee and members of the two DRG Surgery Study Sections in Bethesda, Maryland. The agenda included (a) discussion of the current status and future of training grants, (b) prospective needs for manpower in academic surgery, (c) review of guidelines for academic training in surgery, and (d) program evaluation and site visits. The National Advisory General Medical Sciences Council later approved the recommended changes in the guidelines for the Surgery Research Training Program. These changes, which were not of a basic nature, serve primarily to update the guidelines, recognizing the fact that supplementation of trainee stipends is permitted, that some surgery departments already have sufficient basic science strength to serve as suitable training sites for this aspect of the program, and that some areas not usually regarded as sciences basic to surgery, such as the social sciences, may indeed be appropriate in selected cases.

The Radiology Training Committee held its program directors' meeting in San Francisco. Note was taken of the improving situation with regard to recruitment into the specialty of diagnostic radiology, which would no doubt eventually benefit the recruitment of academic and research-oriented radiologists. The recent establishment of a Specialty Board in Nuclear Medicine (a subdiscipline also under the Radiology Training Committee's purview) has been a contributing factor to improved recruitment. Several problems in the operation of the research training programs were identified. One concerned agreement on the best sequence and arrangement of research and clinical studies; another was the inadequate stipend levels available to trainees; a third related to the difficulty experienced by young graduates of the research training programs in quickly obtaining support to start up their research. A "small grants program" was recommended by the participants.

New Programs

A new program entitled "Minority Access to Research Careers" has been initiated. It consists of faculty fellowships, the purpose of which is to provide increased opportunities for advanced research training for bioscience faculty members of predominantly minority institutions (selected by these institutions). The intent is that faculty fellows will return to their home institutions to initiate independent research programs and to strengthen and further develop undergraduate as well as graduate teaching in the biomedical sciences. Complementary to this type of fellowship is a visiting scientist award, which provides support for outstanding scientist teachers who wish to serve as visiting professors at schools with primarily ethnic minority enrollments. They are expected to strengthen research and teaching programs in the biomedical sciences for the benefit of both students and faculty at these institutions. The first group of 11 faculty fellows and 1 visiting scientist awards were funded during FY 72.

Another new fellowship program was announced in June, 1972--the Medical Scientist Fellowship Program. This program was designed to complement the nine-year-old Medical Scientist Training Program, presently constituting a group of 11 interdisciplinary research training programs of institutional scope. Because of their complexity and high requirements for leadership, the number of these programs has grown only slowly. In order to make available support for combined, integrated medical and graduate education to additional, selected candidates across the nation, the fellowships program was initiated, permitting each school that does not have a Medical Scientist Training Program to nominate two candidates each year. No awards were made in FY 72 because of the late start of this program.

Personnel

The following personnel changes took place. As a result of the death of Dr. Trygve Tuve, Dr. Leo von Euler was appointed Acting Associate Director for Manpower, and Dr. Robert McCauley, Acting Chief of the Research Training Grants Branch. Dr. Myron Sokolowski, who had been responsible for the Biophysics Research Training area, resigned to take a nongovernmental position; Dr. George Hayden was named to take his place. Dr. Edward Hampp assumed the added responsibility of Program Administrator of the Pathology Research Training Program.

Awards and Reviews

Because of the generally increasing commitments for ongoing programs, only three new awards for research training programs were made. This is considerably below what had been the case in previous years, and staff will make every effort to increase the proportion of new awards in the future. The Institute's budget in FY 72 for research training grants was $43,546,000, approximately the same as for the previous year. The total number of grants awarded, however, decreased from 553 in FY 71 to 472 in FY 72.

During the year ending June 30, 1972, 764 fellowship applications were reviewed by the Institute's research training committees. As in the previous year, the review load on a few committees--Biochemistry, Biophysical Sciences, Pharmacology-Toxicology, and Genetics--was particularly heavy.

Research career development award applications are now reviewed by the DRG. In FY 72, 71 of 99 new or renewal applications were approved and funded. In addition, the NIGMS made 271 noncompetitive continuation awards. No new awards are made under the Research Career Award Program, but the Institute is still supporting 32 awardees.

Of the 550 reviewed postdoctoral fellowship applications, 274 were awarded. For special fellowship applications, the corresponding figures were 204 and 138.

The number of individuals supported, and dollars expended, by program area, are shown in the following Table.

FY 1972

NIGMS Training and Fellowship Support

Program Designation	Number of Individuals			Dollars (In Thousands)		
	Training	Fellowships	Total in Program Area	Training	Fellowships	Total in Program Area
Category I						
Anesthesiology	75	2	77	$938	$28	$966
Biometry	181	6	187	1,169	46	1,215
Clinical Pharmacology	17	--	17	286	--	286
Epidemiology	37	1	38	418	8	426
Genetics	502	238	740	2,863	1,732	4,595
Medical Scientist	354	--	354	3,029	--	3,029
Multi-Biological Sciences	435	--	435	2,158	--	2,158
Multi-Medical Sciences	22	--	22	288	--	288
Nutritional Sciences	116	3	119	559	23	582
Radiology	136	4	140	1,968	44	2,012
Subtotal	1,875	254	2,129	13,676	1,881	15,557
Category II						
Anatomical Sciences	279	13	292	1,925	99	2,024
Behavioral Sciences	402	10	412	2,960	84	3,044
Clinical Chemistry	39	6	45	355	80	435
Engineering in Biology & Medicine	295	48	343	1,449	593	2,042
Microbiology	453	48	501	2,419	370	2,789
Pathology	327	9	336	3,427	103	3,530
Pharmacology	496	55	551	3,322	412	3,734
Surgery	127	4	131	2,706	57	2,763
Toxicology	52	5	57	352	41	393
Subtotal	2,470	198	2,668	18,915	1,839	20,754
Category III						
Clinical Anesthesiology	2	--	2	17	--	17
Biochemistry	1,146	172	1,318	5,209	1,366	6,575
Biophysical Sciences	514	123	637	2,756	960	3,716
Medicinal Chemistry	106	21	127	589	187	776
Organic Chemistry	--	68	68	--	403	403
Physiology	452	43	495	2,284	250	2,534
Subtotal	2,220	427	2,647	10,955	3,166	14,121
TOTAL	6,565	879	7,444	43,546	6,886	50,432

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